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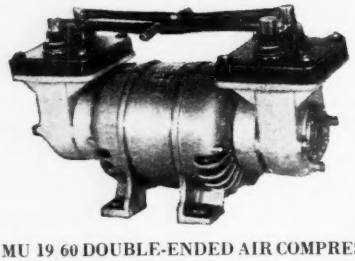

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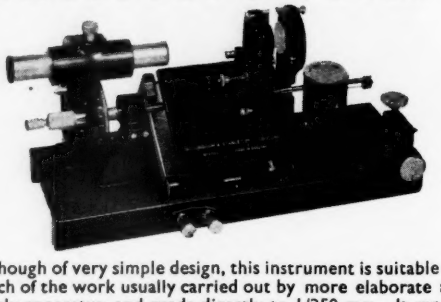

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UNDER-DEVELOPED TERRITORIES AND THEIR PROBLEMS

IN his presidential address to the British Association in Dublin in 1957, Prof. P. M. S. Blackett directed attention to the steadily widening gap between the Western countries and those of Asia and Africa, and advocated a loan of £1,000 million a year, in addition to commercial and short-term government lending either as a free gift or as long-term loans. Britain's share of this would have amounted to £150 million a year. Although the problem of aid to the under-developed countries has since been widely discussed in all its aspects, it was admitted by the Secretary of State for Foreign Affairs, Mr. Selwyn Lloyd, in a broadcast on United Nations Day (October 24), that the rapid rise in the standard of living in the highly developed countries is not being matched by improvements in those less developed. For that reason, he said, the Government will support the work of the United Nations Special Fund to finance large-scale schemes of economic development in such countries, and Parliamentary approval is to be sought to increase five-fold the British contribution to that fund—a contribution at present standing at a million dollars.

It should be noted that Mr. P. Hoffman, director of the Special Fund, has already proposed that the objective should be to double the increase of income a head in the under-developed countries during the next ten years. On the calculations of a report of M. J. Monnet's Action Committee for the United States of Europe, which called on the Common Market countries to adopt a common policy on economic aid to such countries, this would require doubling the amount of capital supplied to the under-developed countries by the outside world, and the figure of 7,500 million dollars annually is about equivalent to 1 per cent of the combined national income of the richer countries—rather less than the proportion represented by the United Kingdom's estimated total contribution to the development of these countries in 1958–59 of £2·15 million. It should, of course, be remembered that, apart from assistance given under the Colombo Plan and under the Colonial Development and Welfare Acts, Britain is contributing in various ways to economic and technical assistance, and in its development fund for overseas territories, the European Economic Community has launched a programme of capital investment in French and Belgian African territories, while, besides its existing massive contribution of some 16,000 million dollars, the United States is being urged by the Senate Foreign Relations Committee to consider a more generous policy to Africa based on the human need to raise standards of living.

One of the biggest obstacles here is the rapid increase in population in those countries, and although the York meeting of the British Association did not give anything like as much attention to the under-developed countries as was done at Dublin, Dr. L. Harrison Matthews's forthright assertion attracted wide attention. In his presidential address to Section D (Zoology), Dr. Harrison Matthews declared that the most serious problem at present facing the human race is the frightening rate of increase of population, especially in the so-called under-developed parts of the world. Past and present experience, he said, gives no grounds for complacency; there will be no room to produce food, or indeed anything else, far less to conserve fauna, if there is one person on every square yard.

This stern warning that uncontrolled increase in population inevitably builds up to a crash, which incidentally included a strong plea that we should welcome any offer of co-operation from the United States in conservation and like matters in Africa and elsewhere, has been strongly supported in a Friday Evening Discourse which Sir Norman Kipping, director-general of the Federation of British Industries, delivered at the Royal Institution on October 23. Sir Norman believes that one of the two hopes for a real advance in the material standards of the under-developed world lies in a simple, cheap and fool-proof method of birth control, which will give India, Egypt and China a breathing space for their output to catch up and run ahead of their expanding populations. His other major hope lies in the development of cheap and simple 1,000 kVa. nuclear-fuelled power stations. Cheap and plentiful power means, among many other things, he pointed out, water which would be pumped and piped from as far as necessary or distilled from the sea.

Sir Norman showed himself much more aware than many speakers on this subject of the magnitude of the effort which the United Kingdom is already making: in personal terms, we are spending on technical assistance annually about 17*s*. 8*d*. a head, and, including private investment, which accounts for about half, our total contribution to the development of the under-developed countries is already rather more than 1 per cent of our gross national product. Even against the 4,500 million dollars of outstanding loans at the World Bank, external assistance to the under-developed Colombo Plan countries in 1957–58 of more than 1,000 million dollars and United Nations Technical Assistance Administration expenditure of some 30 million dollars a year, this contribution is not to be disparaged; but it is right that Britain and other countries should ask themselves, as Sir Norman suggests, whether in scale or urgency such efforts measure up to the task, whether the full significance of the time factor is appreciated, and whether techniques could be improved. The time factor, he recognizes, involves a dilemma, for many of the surest lines of advance involve long-term policies, and he admits that a policy of gradualness is a hard doctrine for many under-developed countries to appreciate.

Much that Sir Norman said has frequently been emphasized in other connexions; for example, notably at the Commonwealth Trade and Economic Conference at Montreal in September 1958, though Sir Norman was concerned more particularly to indicate ways in which industry could render practical help. On one point, he strongly supported the views expressed recently by Mr. M. Ionides before the Royal Institute of International Affairs. Mr. Ionides had argued that Western institutions adapted to societies where the initiative comes from below cannot fully satisfy the needs of a society where the flow of initiative is from the top down. In particular, Western institutions are not organized to tap the immense capital resources in these countries already existing in the form of unused man-power. They can only touch the fringe where a cash economy exists. Frequently, by injecting the large-scale projects, we divert available enterprise and initiative from the much-needed widespread small-scale development, and without providing the means for this basic development. The large-scale project does not even teach the simple methods, or provide the required training in them, or, where it is needed, the organizing ability essential for a start.

Sir Norman Kipping elaborated this factor from a different angle, but was no less emphatic that the impetus must come from above—from the élite few in each country who are in contact with the outside world and have educated themselves in modern techniques, and from those from more advanced countries who have come to trade, to teach or to investigate. By and large, in these countries the springs of individual initiative and resource in economic matters have been lacking, and where there is a new awakening and determination the transition into the modern world is harder and more complicated than it was when European development began. This in itself largely accounts, as Sir Norman remarked, for the tendency to think in terms of the grandiose scheme at the expense of the equally important little schemes. It creates the further problem that the educated few tend, by the very fact of their education, to be cut off from the mass of their own people.

This is an aspect to be remembered in considering the functions and future of the Colonial Development Corporation. It is exactly in such a situation that new institutions are needed, new links with institutions in the more advanced countries, and, generally, a flexible and experimental approach. By its nature, the Corporation has always occupied a position between commercial enterprise and public works financed by taxation. It was enjoined, moreover, to have particular regard for the interests of the inhabitants of the countries in which it worked and not merely to pay its way.

The intertwining of social and commercial ends is naturally liable to cause difficulties and, while under Lord Reith's chairmanship much was done to bring the Corporation's affairs into good shape, in his last two years of office conflicts have arisen over policy and procedure, so much so that Lord Reith complained of frustration, and welcomed the establishment, in April 1959, of an independent investigation into the capital structure of the Corporation under the chairmanship of Lord Sinclair of Cleeve. The Committee interpreted its terms of reference—"to examine the functions of the Colonial Development Corporation within its existing statutory framework; and to consider whether any change in its financial structure is required; and to make recommendations"—as requiring it to determine whether or not the existing financial structure is suited to the fulfilment of the obligations laid upon the Corporation, and, if not, to show why and to state how it could be improved. Accordingly, the Committee began by examining the existing functions of the Corporation in the context of the changing circumstances in which more government funds are being made available both under the Colonial Development and Welfare Acts to the decreasing area of the Colonies, and by means of Commonwealth Assistance loans under the Export Guarantees Act, 1949 and 1957, to the former Colonies but now independent territories.

The wider interest of the Committee's report* arises from this preliminary examination, which appears thoroughly to justify Lord Reith's protests. The territorial scope of the Corporation's activities is diminishing, and particularly in territories which by reason of their stage of economic development would have offered more immediate scope for the Corporation's activities. The Committee unhesitatingly concludes that it is not possible for the Corporation to continue to carry out the purposes for which it was set up, and at the same time meet the obligations laid upon it under the Act, unless there is an immediate change either in its financial structure or in the nature of its activities. If it is desired that the Corporation should carry its existing commitments and, still more, should continue to invest in projects involving a considerable degree of risk, the existing financial structure must be amended to one more compatible with the nature of the Corporation's operations, and the Committee's financial recommendations are to that end.

The detailed recommendations need not be considered here, but it should be noted that although the Sinclair Committee believes that its recommendations will both ensure that the Corporation's true financial position will be more easily deducible from the accounts from year to year, and also help to prevent what would otherwise become an intolerable burden undermining the good the Corporation has done or could do, it points out that no modifications to the financial structure can by themselves transmute what is clearly a very difficult operation into an easy or highly successful one. Parliament must be clear as to what exactly the Colonial Development Corporation is intended to do. But the Sinclair Committee finds the claim valid that the Corporation is unique and essentially distinct from nationalized industries and public corporations

* Report of the Committee of Enquiry into the Financial Structure of the Colonial Development Corporation. Pp. iii+25. (Cmnd. 786.) (London: H.M. Stationery Office, 1959.) 1s. 6d. net.

operating in Great Britain. These are matters rather for the Corporation itself and the Colonial Office, but the Committee leaves no room for doubt that if Parliament wishes the Corporation to continue its efforts to assist economic development in the Colonial Territories, the ordinary rules for payment of interest and repayment of capital must be relaxed; otherwise the Corporation will ultimately be confronted with an annual obligation which it has no reasonable prospect of being able to meet.

The Sinclair Report should thus clarify thinking about both the Colonial Development Corporation and the British contribution to economic development overseas. Specifically, the Committee considers that the Corporation, under the existing Act, should be relieved of liability for interest unpaid at the date when abandonment of a project is approved, and that it should be left to the commercial judgment of the Corporation to decide when to realize on investment, bearing in mind that once a project has become firmly established and is benefiting the economy of the territory concerned, the primary purpose of the Corporation in initiating the project has been achieved. Later, the Committee reiterates the view that the question of accountability as affecting the Corporation is not in practice capable of sharp definition, and suggests that broadly the judgment whether a project is worth while on commercial grounds is primarily one for the Corporation, and the decision whether it is in accord with public policy and within the Act is for the Secretary of State.

In such circumstances it is the more important that questions of Colonial development and welfare and of assistance, whether economic or technical, to the under-developed areas generally should be discussed as free from party political prejudice as possible. The problems are too complex and difficult to be capable of easy solution even when all the facts can be ascertained beyond question. What matters is that discussion should be fully informed, and as free as possible from prejudice, and that programmes of action should be framed to take account of the social and educational difficulties, as well as those of technology, economics and finance. It is obvious that much preparation will be required, even when such discoveries or developments as those for which Sir Norman Kipping pleaded have taken place, before their results can be made available to the under-developed areas. Meanwhile, the more clearly the situation is understood, both in the advanced and the backward areas, and the more resolutely and urgently the United Kingdom and other advanced countries contribute towards meeting existing needs and filling available opportunities, the easier it should be to take full advantage of any fresh advance which will enable mankind to solve this threatening problem of unbalance between population and all its myriad needs and the material resources in food, in fuel, in energy and mineral wealth to satisfy them. Sir Oliver Franks remarked early this year that the under-developed countries cannot be free to embark upon development programmes regardless of their own resources, and then expect the industrial countries to underwrite them to an unlimited extent; and, on the other hand, for the developing countries to have any chance of raising their standards of living they must undertake capital development on a scale which is bound to make their demand for imports outstrip their ability to pay in exports for many years to come, quite apart from the population question. There, however, lies the real problem, and if the gap in living standards is to be closed and all its explosive possibilities removed, both understanding and a sense of urgency will be needed; the situation requires human sympathy as well as economic insight and the wise use of all instruments that come to hand.

NUMBERS OF PEOPLE

The Study of Population

An Inventory and Appraisal. Edited by Philip M. Hauser and Otis Dudley Duncan. Pp. xvi+864. (Chicago: University of Chicago Press; London: Cambridge University Press, 1959.) 112*s*. 6*d*. net.

THE counting of people is almost as old as man himself. Concern about numbers is old but not so old. The study of population as a narrow field of formal scientific inquiry goes back only three centuries. The recognition of the interdisciplinary character of the more embracing study of population in relation to space and resources is recent and not yet sufficiently widespread. An account of historical development linked with and supporting both a contemporary assessment of the status of demography as a science and an evaluation of the possibilities of its integration with other scientific disciplines has been needed for some considerable time. This need has now been met. Such a project is a formidable undertaking and the size of the resultant volume is also formidable. One wonders whether it was necessary to have separate chapters on the development of demography in different countries except as a concession to national pride. It would have been better to have a world review of significant developments shown in relation to the social and economic pressures which stimulated them. The editors, too, have spread themselves unduly in the introductory chapters. But it is all interesting and well written; and more ruthless editing would not have made the book much lighter.

Undoubtedly the general pattern of the book is right: four chapters on demography as a science; nine chapters covering historical development on a world-wide scale; twelve chapters on different subfields in demography; seven chapters on population studies in various disciplines (genetics, human ecology, sociology, anthropology, economics, geography); and, surely a blessing in so large a volume, the 'overflow and conclusions' form the subject of Chapter 1. The historical chapters make fascinating reading. The authors have succeeded in bringing to life the significant personalities and the raging controversies (of which the Malthusian is but one) of earlier days. The treatment of British demography by Prof. E. Grebenik is balanced and fair. It is sadly true that while considerable developments of technique were made by actuaries throughout the nineteenth century, formal teaching in British universities has lagged. This is mainly due to the lack of career

outlets for demographers, the incentive to specialize being correspondingly small. Perhaps, however, there should be less specialization. In those vast areas of the world where population growth presses so menacingly upon under-developed economies, pure demographers are less in demand than those with a wider and multidisciplined approach, an understanding of the subtle inter-relationships between demographic, geographical, ecological, social and economic factors. Just how wide the field of knowledge must be is excellently illustrated in the closing chapters of this symposium. Everywhere knowledge is incomplete. Information concerning land and other resources, the effect of locational factors, genetic changes and biological influences, cultural forces, and the manner of community expansion and reorganization—all this now concerns the population student.

If any part of the book is to be criticized, it is the form and content of the middle chapters. This is not a question of factual or expository deficiency, for authorship is of a very high standard. One gets the impression of a little too much erudition, particularly too much factual content, and not enough concentration upon methods of measurement. The chapter on population distribution criticizes the descriptive and one-sided character of contemporary studies, but appears to favour methods of multiple regression analysis which succeed only to the extent that they are descriptive, and are in disrepute because they have built-in hazards of interpretation. It ignores the volumetric approach of the geographer. The fertility chapter affords little space to the index problem in measurement and concentrates more on the subject of fertility regulation ; on the other hand, the chapter on population replacement is largely confined to methods of arithmetic with barely enough interpretative caution. But it must be admitted that it is always difficult to decide how much space to allot to explaining how to measure and how much to discussing the essential character of the events that are being measured. The book is claimed to be an encyclopædic summary. It is a difficult feat of editorship to prevent this from being a contradiction in terms and the editors are to be congratulated on a very high measure of success. This is more than a reference book ; it will attract sustained study.

B. Benjamin

STERIC EFFECTS IN ORGANIC MOLECULES

Steric Effects in Conjugated Systems

Proceedings of a Symposium held at the University, Hull, 15–17 July, 1958, by the Chemical Society. Edited by Dr. G. W. Gray. Pp. viii+181. (London : Butterworths Scientific Publications ; New York : Academic Press, Inc., 1958.) 30*s*.

THIS relatively inexpensive book is a useful addition to the small number of books concerned with steric effects in organic molecules. It contains the fifteen original papers, together with 44 pages of ensuing discussion, which were read at the symposium held in Hull during July 15–17, 1958.

A lucid historical survey by E. E. Turner was followed by seven papers in which the relationship between the light absorption and the conformation of conjugated systems was discussed. The theoretical treatments of C. A. Coulson and of W. F. Forbes were accompanied by papers by G. H. Beaven, C. C. Barker, and by E. S. Waight and R. L. Erskine, incorporating detailed measurements of the spectra of numerous alkyl diphenyls, di- and tri-phenylmethane dyes, and conjugated carbonyl compounds, respectively. Certain spectral data were related to rates of reaction and the pK_a by B. M. Wepster, though the author pointed out that this approach is not universally applicable.

Attempts to evaluate steric and electronic effects with the aid of kinetic data were described in four papers. The rates of nucleophilic aromatic substitution, of the solvolysis of aralkyl halides, of the acid-catalysed H–D exchange in aromatic hydrocarbons, and of a variety of reactions involving *ortho*-substituted compounds were discussed, respectively, by N. B. Chapman, H. C. Brown, G. Dallinga *et al.*, and A. C. Farthing and B. Nam.

The remaining papers illustrated the application of other physical methods. A paper by E. A. Johnson on the gas-liquid chromatography of alkyldiphenyls provided an interesting complement to that by Beaven. Contributions by J. W. Smith and by G. W. Gray described dipole moment measurements and observations on mesomorphism, respectively.

In summary, this collection of original papers embodying review material and some discussion provides a valuable survey of contemporary studies concerning the conformations of conjugated molecules.

G. Eglinton

COLORIMETRY

Colorimetric Methods of Analysis, including Photometric Methods

Vol. 2A. By Dr. Foster Dee Snell and Dr. Cornelia T. Snell, assisted by Dr. Chester Arthur Snell. Pp. x+793. (New York : D. Van Nostrand Company, Inc. ; London : D. Van Nostrand Company, Ltd., 1959.) 112*s*. 6*d*.

THE volume under review supplements the data given in the third edition (1948–54) of Volume 2, and includes all the important publications which have appeared prior to January 1956. In dealing with such a vast compilation, now five volumes, the authors cannot from their personal experience recommend any particular method, and the question arises as to whether treatises such as this have a universally useful function. The answer appears to be that this treatise has been compiled by a team of experienced abstractors who can consult their own analytical department, and who are thus well able to judge whether a method merits recording or not.

The scheme adopted throughout the volume is both logical and helpful to the reader. Each chapter is headed by a brief description of new and useful additions to the methods described in the previous edition of Volume 2. Where the method is the result of collaborative study by a number of laboratories it is frequently given almost in full. Thus the reader can proceed with such a method in confidence, knowing that it has been tested in a number of independent laboratories, and has justified its publication by a representative body of experts. While this results in recording methods which have proved sound, these methods may not be the best available even shortly after publication, since collaborative studies between widely separated laboratories are necessarily very time consuming.

One of the most useful new features in the volume is the prominence given to the use of flame photometry and fluorimetry. As stated in the preface, the improvements in burners and the application of photomultiplier tubes have greatly increased the sensitivity of the flame photometer, and improved tubes that of the fluorimeter. It would have been helpful if the general principles of the improved methods both for flame photometry and fluorimetry could have been described as a separate chapter, but presumably these will follow in a supplement to Volume 1. Without such help it may be puzzling to understand why on page 471 an oxy-acetylene burner is recommended for the determination of the alkali metals, but a propane-air burner on page 475. Much depends on the concentration of interfering elements and on the choice of internal standards. While such standards are desirable, their use may be attended by difficulties, since the optimum burner temperature for development of the colour of the test element may be far removed from that of the standard. What is required is not merely high sensitivity, but also uniform light emission with varying temperature. As a useful alternative to flame photometry for the determination of lithium, the application of fluorimetry is described, the sensitivity being such as to make as little as 0·005 mgm. determinable in the presence of limited amounts of sodium and potassium.

The amount of new material described in Volume 2A is astonishing, bearing in mind the short interval of time which has elapsed since the publication of Volume 2, and the original treatise of four volumes cannot be considered complete without Volume 2A. The references have been carefully chosen from an extremely wide selection of the world's scientific journals, and unlike some U.S.A. publications have no bias in favour of American work. J. KING

STATISTICAL METHODS

Principles of Statistical Techniques

A First Course, from the Beginnings, for Schools and Universities. By P. G. Moore. Pp. viii+239. (Cambridge: At the University Press, 1958.) 22*s*. 6*d*. net.

IN the Preface to this excellent text-book the author claims that it is ". . . an attempt to put across the main principles of statistical methods . . .". This claim is abundantly justified. The book is readable from start to finish, and only in Chapters 10 and 11 (dealing with tests of significance) will more than normal concentration be needed, even if the reader is approaching the subject for the first time. The book will be a valuable addition to every grammar-school library. Many sixth form pupils, especially those reading geography or economics, will derive great benefit from a careful study of it; in the field of general studies, however, it will appeal to a much wider audience so that merely a single reading will help towards a clear understanding of the part which observation and sampling now play in pure research and in industrial investigations.

The author's style is attractive and the reader is led easily through chapters on the collection, tabulation and representation of data, frequency distribution and dispersion, to a careful study of the power (and the weaknesses) of sampling. The application of the binomial series and time series, together with a rather more technical consideration of significance, bring the student to a treatment of regression. The printing, tabulation and graphs have the excellence normally associated with the Cambridge University Press and there are few misprints. Important among these is the misuse of $\sqrt{npq}$ for $\sqrt{(npq)}$ in a number of places, and the same error is found (p. 160) in the formula

$$s=\sqrt{\frac{1}{n}\Sigma(x-\bar{x})^2}$$

At the end of each chapter there is a good set of exercises, but the value of these is greatly reduced by the absence of answers. It is to be hoped that the author will remedy this by a pamphlet until another edition is available. With this one serious reservation the book may be recommended warmly to anyone who needs to know, either for practical purposes or as a matter of general interest, how the analysis of observations can be accomplished. W. J. LANGFORD

PERCEPTION AND LEARNING

Dynamics of Behavior

By Prof. Robert S. Woodworth. Pp. x+403. (London: Methuen and Co., Ltd., 1958.) 36*s*. net.

PROF. WOODWORTH began his scientific career some sixty years ago with an admirable little book on movement, written in French. He afterwards worked with Sherrington on pseudo-affective reflexes and seemed well set to develop American psychology along experimental and physiological lines. Yet leadership in research somehow eluded him. The initiative passed to others, in particular Lashley, and it was left to Prof. Woodworth to provide the text-books on which successive generations of psychology students in the United States have been nourished. Plain fare, perhaps, but wholesome.

The present volume has its origin in the author's "Dynamic Psychology", first published in 1918. But it is far less a revision of the earlier book than the substance of a lecture course based upon it and progressively developed by Prof. Woodworth over the past forty years. The content ranges from reflexes to reasoning, the greater part being concerned with perception, motivation and learning conceived in terms of biological adaptation. Although the eclectic point of view will be familiar to readers of Prof. Woodworth's earlier texts, the present volume has the advantage of bringing together a great deal of experimental material, much of it recent, relevant to the general analysis of behaviour. It also provides a useful guide to the many, and often conflicting, theories of learning which have been evolved by American psychologists during the past thirty years. This is the kind of thing which he can do very well.

Prof. Woodworth demonstrates that his capacity to expound contemporary points of view in psychology has been in no way dimmed by advancing age. At the same time, his account of modern work perhaps suffers from the lack of any well-defined point of view of his own. Had the author held fast to his Sherringtonian origins and attempted to develop a theory of behaviour along evolutionary and physiological lines, it is possible that some real clarification might have been achieved. As it is, we have to make do with a somewhat ill-assorted conglomeration of facts and theories, imperfectly related one to the other, and the whole scarcely warranting treatment as a scientific discipline. Although it may be objected that this picture truly reflects the existing state of psychology, it might seem a pity to enshrine it in a formal text.

A contribution more limited in scope, tauter and more incisive in argument, would have served the purpose better.

The author's style betrays a certain trans-Atlantic homeliness that may cause some elevation of English eyebrows. Thus the reader is urged to "get acquainted with gravitation" much in the same way as he might be advised in other quarters to make friends and influence people. It may also be surmised that the prevailing emphasis upon "outgoing human tendencies" owes more to American culture than to the scientific study of man.

It is encouraging to note that Prof. Woodworth retains something of his interest in the European scene. Although ethology appears to have escaped him, British work in experimental psychology is exceptionally well represented. O. L. Zangwill

NUCLEAR ENERGY AND ANALYSIS

Analytical Chemistry

Vol. 1. Edited by M. T. Kelley. (Progress in Nuclear Energy, Series 9.) Pp. vi+372+xii. (London and New York: Pergamon Press, 1959.) 105*s*. net.

THIS volume consists of a collection of some of the papers of interest to analytical chemists which were presented at the Second International Conference on the Peaceful Uses of Atomic Energy held at Geneva in 1958. These papers have already been made available in Vols. 3, 19 and 28 of the records of the Conference published by the United Nations, and this new book simply presents the material in a single volume of a more convenient size. The papers have been arranged in five groups as follows: "Reactor Applications", "Activation Analysis", "Spectrographic Technique", "Industrial Applications", and "Health Physics". All papers are in English with the exception of two which are in French. The reason for not publishing a complete volume in English is not clear, especially since these two papers are available in English in the United Nations publications.

The first group of papers contains one giving the Harwell experiences in analytical chemistry work connected with reactors. Two excellent review papers are also included, one dealing with most aspects of the analytical chemistry of thorium and the other on chemical methods for the determination of uranium in ores and associated materials. In the activation analysis section, much information is given about Canadian and United States experiences with this valuable technique for trace determinations, especially when used in conjunction with γ-spectrometry. A notable omission from this section is the excellent paper by Smales and co-workers reporting British experiences with this technique; this paper is available in Vol. 2 of the United Nations publications.

Other papers of note in the remaining sections deal with the use of infra-red spectroscopy for determinations connected with the use of heavy water in atomic energy work, and the use of non-destructive X-ray spectroscopy for the analysis of reactor fuel components. Fassel's spectrographic method for the determination of gases in metals is also described. In the section on "Industrial Applications", developments in the analytical services given to the programmes of the Industrial Groups of the U.K. Atomic Energy Authority are described. Other papers deal with the use of radioisotopes in Japanese industry and the determination of tungsten in steels by employing the back-scattering of β-particles.

The editing of this book could have been better and the index should have been more detailed. A price of 5 guineas for a book of this type is very high.

G. W. C. Milner

NORTH AMERICAN MAMMALS

The Mammals of North America

By Prof. E. Raymond Hall and Dr. Keith R. Kelson. Vol. 1: Pp. xxx+1–546+79. Vol. 2: Pp. viii+547–1083+79. (New York: The Ronald Press Company, 1959.) 35 dollars the set.

THE North American continent has a rich mammal fauna (995 species according to this compilation), and it has also a relatively high population density of mammalogists, among whom Prof. E. Raymond Hall is one of the best known. With Dr. K. R. Kelson, he has completed the enormous task of bringing together the most up-to-date facts and opinions about the taxonomy and distribution of North American mammals in an excellent production.

The costs of preparing the manuscript, with its 500 maps, 553 figures, and numerous line drawings, must have been high, even by New World standards. The completed work is a monument, not only to Prof. Hall's ability as a mammalogist, but also to his talent for raising funds from official sources.

Prof. Hall's attitude to the law of priority in nomenclature is regarded by some people as admirable, and by others as intransigent. It is certain that the vast majority of zoologists will regret his following Hershkovitz in calling the Virginian deer *Dama*, in the face of the request by the International Commission on Zoological Nomenclature to 'freeze' the situation pending its decision. If the change is accepted (and here we find it in what will be a standard work for generations), it means that the European fallow deer, known to all as *Dama*, must be called *Odocoileus*. The Commission will most probably decide to use its plenary powers to preserve stability in this instance. Likewise, the Commission is considering the question of the availability of Oken, 1815–16, "Lehrbuch der Naturgeschichte", and Morrison-Scott has asked it to validate, among others, the well-used names *Citellus*, *Vulpes*, *Panthera* and *Pan*. Hall and Kelson have rejected Oken while the matter is still, so to speak, *sub judice*, and have made some regrettable changes.

One is surprised to find Merriam's 78 species of bears still recognized, but this directs attention to the fact that the authors have not attempted to revise questions of status, but have put forward the views of the most recent workers on a group, even when those views are patently unsound. Many changes in taxonomic status will occur, and many subspecies recognized here will disappear, but here they are, all clearly listed, and it is far preferable to summarily dismissing them as synonyms without explanation.

Everyone will be able to find something to disagree with in such a large work as this, but on the whole the work will be welcomed by every worker on North American mammals. It is essentially for the professional, and outside the United States will have limited sales, but purchase by every university is obligatory. For zoologists in other countries the distribution maps alone are worth the price.

Peter Crowcroft

Synthesis of Linear Communication Networks

Vols. 1 and 2. By Wilhelm Cauer. Second edition edited by Wilhelm Klein and Franz M. Pelz. Translated from the German by G. E. Knausenberger. (McGraw-Hill Electrical and Electronic Engineering Series.) Pp. xxxvi+866. (London: McGraw-Hill Publishing Company, Ltd., 1958.) 151*s*.

THE classical work of W. Cauer on network synthesis was first published in 1941 and has only now appeared in an English translation. During the fourteen years since the death of Cauer there have been many developments in network theory which have been treated at length in a number of recently published text-books. But Cauer's work cannot be regarded as out of date nor is his book in competition with these modern American texts. Rather is it one of those permanent collections of work from which modern theory derives.

The first part of the book contains a thorough treatment of one-terminal pair and two-terminal pair networks along lines which have now become familiar. The image-parameter network is treated in very great detail and considerable attention given to the design of filters. In the second part, Cauer treats reactance networks in general and expands the theory of frequency band-separation networks, a subject neglected in most other works.

An attempt is made to bring Cauer's work up to date by the inclusion of a 44-page appendix outlining the recent advances in network theory and presenting a classified bibliography containing about 450 references.

Advanced Analytical Chemistry

By Prof. Louis Meites and Prof. Henry C. Thomas. With a chapter on Infrared Radiation by Prof. Robert P. Bauman. Pp. xi+540. (London: McGraw-Hill Publishing Company, Ltd., 1958.) 69*s*.

MODERN thought in analytical chemistry centres largely in the field of instrumental or physico-chemical methods; this is reflected in the ever-growing number of text-books dealing with this subject, either alone or as part of a study of 'advanced analytical chemistry'.

The present book, written by two authorities in the field of electrochemistry (as applied to analytical chemistry), is basically the outcome of seven years experience in teaching undergraduate and graduate students at Brooklyn Polytechnic and the University of North Carolina. On reading this book one can only envy these students their courses of instruction.

The book is divided into twelve formal chapters which take the reader from a short but excellent discourse on the nature of analytical chemistry to a similarly well-written account of ionic equilibria in solutions and then to a series of chapters dealing with selected methods of instrumental analysis. These include *p*H and redox potentiometry, conductometry, polarography and amperometry, electrolytic methods, spectrophotometry (visible, ultra-violet and infra-red), radiochemistry and finally ion exchange and chromatography. This part of the text is concluded by a chapter on the development of an analytical method; this unusual inclusion in a student text is commendable in that it illustrates admirably the nature of analytical chemical research. A number of appendixes then interrupt the text and these are followed by a longish (116 pages) section devoted solely to about ninety practical applications of the methods discussed earlier.

Unlike many books on this subject, the present one gives prominence to the theory of the methods, although unifying this theory with the practical exercises. The instruments themselves are subordinated to the theory underlying their use and it is indeed refreshing to find that the student is taught the essentials of a method so well, often on simple instruments, that when he encounters a more elaborate instrument he can "confidently be referred to the manufacturer's instruction book for information on how to turn the knobs".

Much could be said about this book, but in the end the conclusion would be the same—an excellent book, and well worth study not only by students but also by research workers in analytical chemistry.

W. I. STEPHEN

The Structure and Properties of Porous Materials

Proceedings of the Tenth Symposium of the Colston Research Society held in the University of Bristol, March 24th–March 27th, 1958. Edited by D. H. Everett and F. S. Stone. Pp. xiv+389. (London: Butterworths Scientific Publications; New York: Academic Press, Inc., 1958.) 60*s*.; 12 dollars.

THIS is a collection of papers from the tenth Colston Research Symposium held in Bristol last year. Its scope may be indicated by listing the authors: Rideal; Barrer; Wynne-Jones; Kington; de Boer; Everett; Dacey and Fendley; Flood; Wiggs; Kiselev; Foster and Thorp; Schofield; Mougey, François-Rossetti and Imelik; Kavtaradze; Rance; King; Honeybone and Harris; Preston. As the names indicate, the authors' interests range from almost pure physics to biology. The verbal discussions are particularly interesting, and show a fair degree of cross-fertilization. They have been taken from tape recordings, and the temptation to over-edit has been avoided. A few staccato exchanges read like verbatim reports of cross-examination, and the flavour of the individual contributions has been retained. The reviewer approves of this, though he can conceive that this approval will not be universally shared.

Demographic Yearbook 1958

(Special Topic: Marriage and Divorce Statistics.) Pp. viii+541. (New York: United Nations; London: H.M. Stationery Office, 1958.) Cloth, 8 dollars; 57*s*.; 34 Swiss francs. Paper, 6.50 dollars; 46*s*.; 28 Swiss francs.

THE "Demographic Yearbook" of the United Nations continues to provide the basic data for the population of the world. The 1958 issue which has recently been published shows the annual rate of increase of the world's population over the period 1950–57 to be sixteen per thousand. Central America, with a rate of twenty-six per thousand, is increasing fastest; North-West Europe, with a rate of six per thousand, shows the slowest rate of increase. Figures of births, deaths, birth-rates, death-rates and expectations of life are again included, but the special topic dealt with in this issue is the statistics of marriage and divorce. Data on marriages, the ages of persons married, singly and in combination, and marriage- and divorce-rates are given for the various countries. There is also an introductory chapter in which the uses of nuptiality statistics are discussed.

The volume continues the standard of excellence that one has come to expect from its predecessors.

SPIRAL AND OTHER GROWTH FORMS OF SYNTHETIC DIAMONDS: A DISTINCTION BETWEEN NATURAL AND SYNTHETIC DIAMOND

By Prof. S. TOLANSKY, F.R.S., and Dr. I. SUNAGAWA
Royal Holloway College, University of London

IN our laboratory, we have, during the past fifteen years, examined the surface microstructures of many hundreds of fine natural diamonds, mainly octahedra and dodecahedra, using precision optical techniques. In view of the importance of the screw dislocation theory of crystal growth, we have sought diligently for spirals on these diamonds but we have never yet found a single indication of the existence of a spiral on any of the several thousand faces examined. Nor, so far as we know, has anyone else. Our techniques are sufficiently sensitive to reveal spiral growth sheets of height even less than 10 A. We find with natural crystals that octahedral faces invariably exhibit growth trigons, dodecahedral faces show striations, networks or elevated disks and cubic faces are extremely rough. We are quite categorical about never yet having found a growth spiral on diamond; indeed, the only near resemblance to such was experimentally identified as a percussion mark, simulating in its crack directions a crude resemblance to a spiral.

We have recently acquired some specimens of the micro-diamonds made synthetically by the General Electric Company of Schenectady, some kindly loaned by Dame Kathleen Lonsdale, of University College, London, and some by Dr. Custers of Johannesburg. These are being subjected to a detailed examination, using both interferometry and a particularly sensitive phase-contrast microscope, from Japan, which is at our disposal. The new characteristics we describe here are shown by both samples. They have not been reported before in the very extensive literature on natural diamonds. We describe here three new characteristics shown by these synthetic diamonds.

(1) There are occasional cubic faces on the synthetic diamonds which have a highly perfect smooth optical finish. Fig. 1 (× 480) shows such a cubic face photographed with the sensitive phase-contrast microscope. It should be mentioned that this microscope has successfully revealed steps on other crystals which we have established interferometrically to be as small as some 2·5 A. It follows, therefore, that the relative uniformity of the phase-contrast picture is evidence of an exceptionally smooth flat perfection of surface, a perfection locally approaching uniformity of the order of lattice dimensions. Such smooth perfection on cubic faces is quite unknown on natural diamonds, although we have found such perfection occasionally on one or two highly perfect natural octahedral faces.

(2) Quite a number of synthetic diamonds reveal a new form modification. They are typical dendrites. These dendrite characters we find in two distinct ways. On most diamonds they appear as network (dendritic ?) overgrowths (see Fig. 2, × 480). Slip effects are also clearly visible. We believe the dendritic markings to be diamond since both strong heat and chemical reagents do not affect them. However, far more striking is the occasional occurrence of complete dendritic crystals, typical examples of which are shown in Fig. 3 (× 75).

In connexion with these dendrites attention is directed to the fact that they most closely resemble in shape some germanium dendritic crystals in our possession, and, of course, it will be recalled that the germanium crystal has the diamond structure.

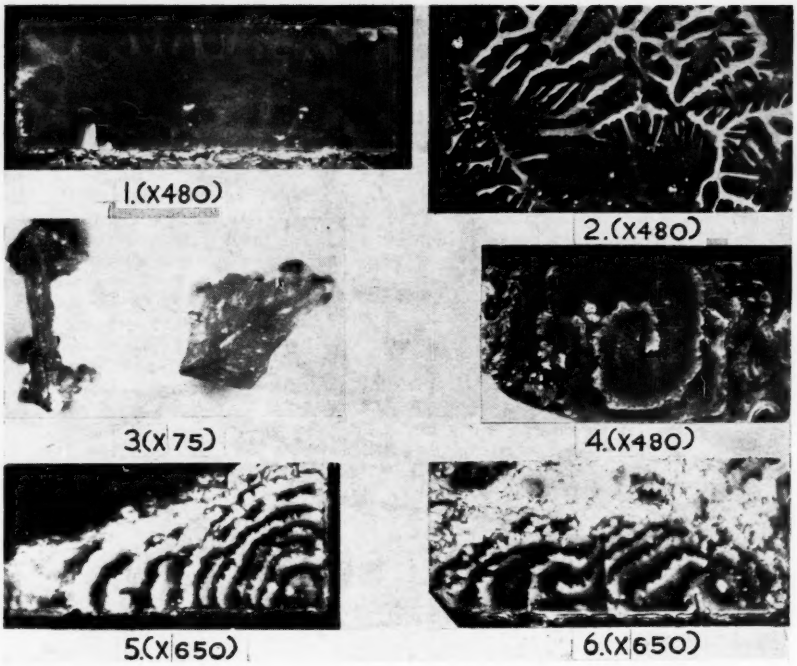
So far as we know, completely dendritic crystals such as we find here in synthetic diamond have never been reported for natural diamond, so that we have a second distinction between the synthetic and the natural crystals.

(3) Our most notable observation has been the discovery of several spiral growths on quite a number of the good-quality cubic faces of the synthetics. Fig. 4 (× 480) shows our finest example, taken with the phase-contrast microscope. On the original it can be clearly seen that the spiral arms are kinked, but that the separate components of the kinked arms are rectangular; the units are parallel to the edge between cubic and octahedral faces. This establishes that the spiral really does belong to the diamond, for if it were a foreign overgrowth it would be too remote a possibility to find something completely epitaxial in lattice to fit the cubic face of the diamond. In any event we cannot remove the spiral and are quite satisfied that it is really diamond.

From our familiarity with comparison of our phase-contrast pictures with our multiple-beam interferograms on other crystals we can make a reasonably confident estimate of the step height of the spiral. We estimate this to be of the order of some 100 A. The quality of the surface between the spirals is very good, and on our originals we see close evidence of a secondary much weaker spiral originating from the same dislocation region.

The kinking of the spirals is reminiscent of those we have seen at times on organic crystals and bears no resemblance to the silicon carbide spirals so often recorded in our laboratory. Their character is essentially cubic. The setting on our phase-contrast instrument tells us that these are true hillock spirals, the centre being the high region, as is to be expected.

A number of spirals have been found on different crystals, and it would appear that most of them favour originating near a corner of the cubic face. Fig. 5 (× 650) is one such corner spiral and Fig. 6 (× 650) is another. Fig. 6 exhibits a further interesting topographical characteristic, in that there are quite clearly shown a number of distinct slip lines crossing the spiral arms.



Two matters appear to call for comment. The first is that these micro-crystals were grown rapidly, which is probably far from the case of the natural diamonds. A second, and perhaps more important, point is that, according to Dame Kathleen Lonsdale, this type of synthetic diamond may have up to even 3 per cent impurity included, in the form of metal carbide. It may well be that the inclusion of so much metal carbide in the lattice is responsible both for the appearance of the dendritic form and also for the appearance of the spiral dislocation, since neither of these features appears in the natural diamond.

Having made these observations, we secured for examination, through the courtesy of Mr. R. G. Mason, of Industrial Distributors (London), Ltd., no less than fifty well-formed natural cubic diamonds. All were Congo boarts, and the idea behind this was that we wished to examine natural cubes which clearly contained impurity too. The colours were greys, browns and blacks. Many of the 300 faces available are hopelessly too rough for precision optical examination, but some of the smaller stones were of reasonable surface quality. We have found no trace either of (*a*) a high-quality surface or (*b*) spirals.

Thus we still confirm the complete absence of any growth spirals on natural diamond faces, whether octahedral, dodecahedral or cubic. We therefore incline strongly to the view that both the spirals and dendrites reported here are due to some special causes distinguishing the synthetic from the natural diamond. This may be either due to different conditions during formation, or alternatively it may be due to metal carbide inclusion.

A VIVIPAROUS DEEP-SEA JELLYFISH

By Dr. F. S. RUSSELL, C.B.E., F.R.S.
Marine Biological Association, Plymouth

ON a cruise off Santander, to collect deep-sea animals for Dr. S. K. Kon and Dr. L. R. Fisher, of the Development Commission Fisheries Research Biochemical Unit, R.V. *Sarsia* brought up on October 18, 1959, in a deep haul with the Isaacs Kidd pelagic net (3,300 metres of wire out) a very remarkable jellyfish (Fig. 1).

This is a deep brownish-red and plum-coloured semaeostome scyphomedusa measuring about 50 cm. across the umbrella. It has four simple mouth arms each more than 1 metre in length. It apparently has no tentacles. The stomach is about 25 cm. in diameter and from it issue 17 or 18 straight radial canals alternating with a number of canals joined to form networks and running for 6–7 cm. towards the umbrella margin, after which they all break up peripherally into a much finer anastomosing network. The margin is somewhat damaged but there are indications of a continuous ring canal. There are four inter-radial gonadial pores each 1·5 cm. in diameter.

But the most remarkable feature is that the medusa is viviparous. Each of the four gonads

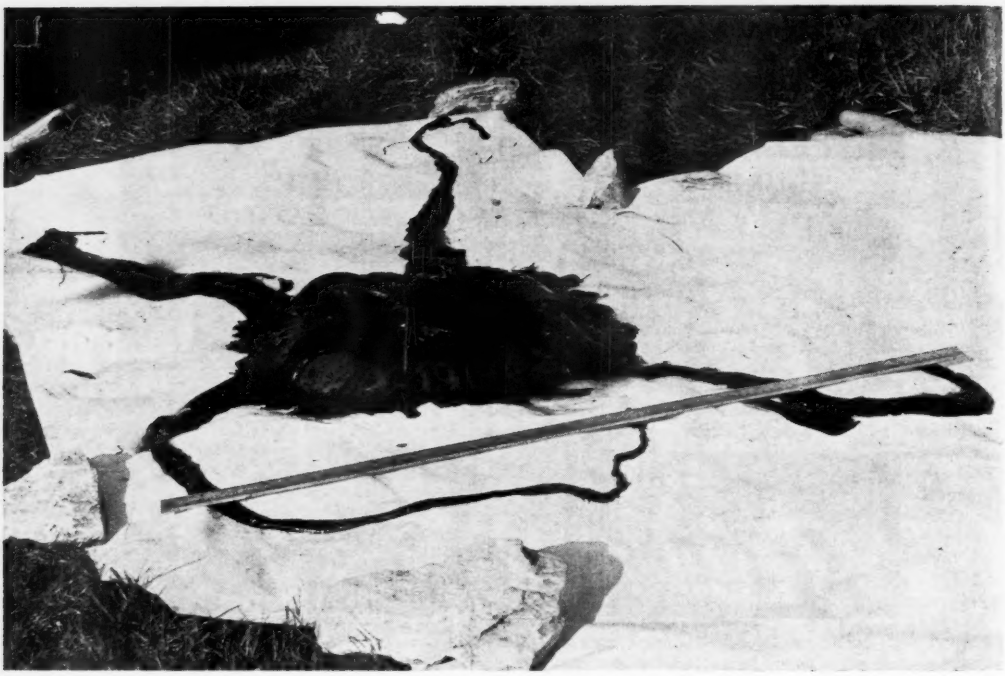


Fig. 1. *Stygiomedusa fabulosa* n.g., n.sp. There is a metre rule in the foreground

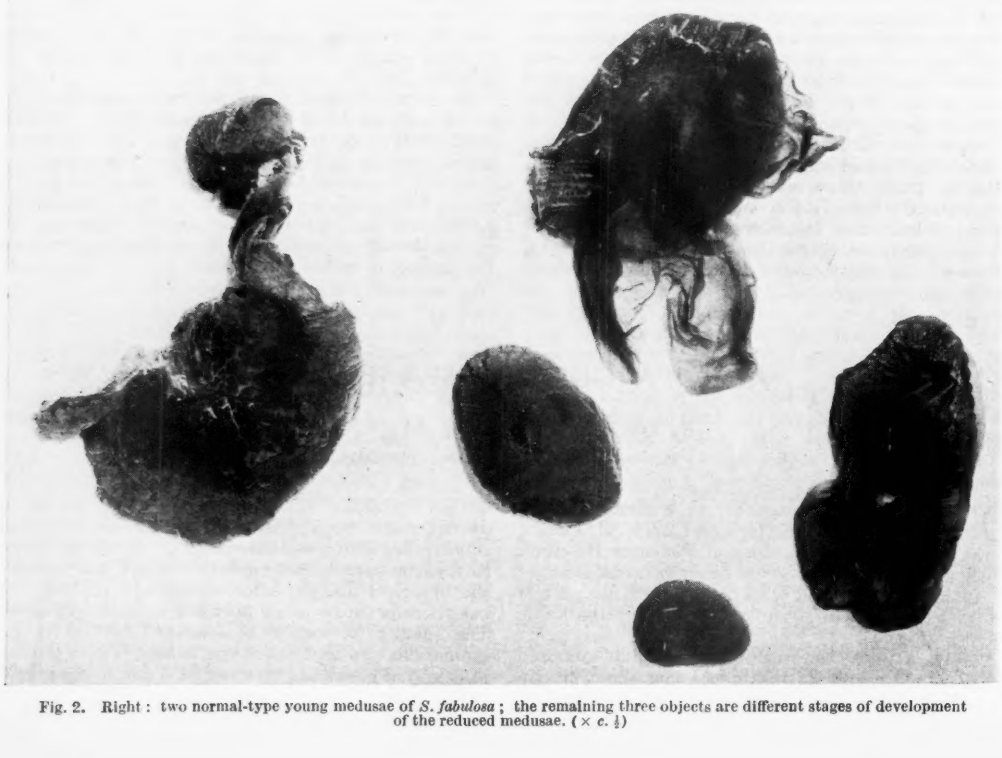


Fig. 2. Right: two normal-type young medusae of *S. fabulosa*; the remaining three objects are different stages of development of the reduced medusae. (× *c.* ½)

bears several white ovoid bodies attached to its wall. These are elongated and narrowing sharply distally, and they vary in size from 1·0 cm. × 0·5 cm. to 5·0 cm. × 2·5 cm. One still larger, 7·5 cm. × 5·0 cm., contains a large developing medusa. There are two fully developed young, each about 10 cm. in diameter. These are perfect except that each has a large hole in the apex of the umbrella, presumably where they were, perhaps prematurely, separated from the stalk. They have 20 or 21 marginal sense organs and about sixty marginal lappets. Neither has any tentacles and this confirms their absence in the adult. The mouth arms are well developed and broad and filamentous. The developing gonads are already fully visible. There are many canals issuing from the stomach, all forming a very branched network of canals around the periphery as in the adult. This network runs into a continuous ring canal round the margin.

There are also four objects which I thought at first to be developing young broken away from their stalk. Two of these appear as rather featureless lumps of jelly with a honeycombed pattern. In one there is the obvious beginning of the development of a lobed umbrella margin and there is one which has developed into a flat disk-like object about 10 mm. in diameter (Fig. 2, left). This is evidently a reduced medusa, because it has ten marginal sense organs, but no mouth. The nature of these bodies must await further examination.

There is no doubt that this is an entirely new semaeostome scyphomedusa coming near the Ulmaridae but differing in the absence of tentacles. I am naming it *Stygiomedusa fabulosa* n.g., n.sp., and I hope later to be able to give a more detailed description.

The position of capture was approximately lat. 44° N., long. 3° 45′ W. It cannot be said from what depth the specimen actually came, but from its coloration and its habit of viviparity it must certainly normally live at great depths.

I would like to thank Capt. C. A. Hoodless and all those on board R.V. *Sarsia* for the care they took in preserving this specimen and bringing it back safely to the laboratory, and Mrs. E. A. Peace for taking the photographs.

CONSERVATION IN THE SCOTTISH HIGHLANDS

A SYMPOSIUM on "Conservation in the Scottish Highlands" was held by the Scottish Branch of the Institute of Biology during September 29 and 30 in Glasgow, followed on October 1 by an excursion to the Black Wood and Moor of Rannoch. The following papers were read: "Problems of Conservation in the Highlands", by Prof. W. H. Pearsall (Nature Conservancy); "Geology of the Scottish Highlands and Islands", by A. C. MacGregor (Geological Survey of Great Britain, Edinburgh); "Soil Conditions in the Highland Areas", by R. Glentworth (Macaulay Institute for Soil Research, Aberdeen); "Soil Erosion in Scotland", by R. M. Gorrie (ex-Indian Forest Service, Edinburgh); "The Role of Bracken in Highland Ecology", by Prof. K. W. Braid (Aberdeen); "Scottish Soils in Relation to Afforestation", by W. O. Binns (Macaulay Institute for Soil Research); "Peat Afforestation", by M. V. Edwards (Forestry Commission); "The Effect of Conservation Practices on Animal Distribution", by W. J. Eggeling (Nature Conservancy); and "Conservation of Sheep Grazing", by R. F. Hunter (Hill Farming Research Organization). The present article is based on these papers and on the discussions which followed them, and the views expressed are not necessarily those of the reviewer.

The Scottish Highlands comprise, in a geological sense, that part of the country to the north and west of the Highland Boundary Fault. This area, in addition to the traditional crofting counties, includes more prosperous farming counties such as Aberdeenshire, Moray, Nairnshire, Banffshire and parts of Perthshire. It is in the crofting counties that the problems of depopulation, social decay and land degradation have reached serious proportions, and it is these that are causing concern.

When considering the Highlands, and conservation there, it is important first to pose the question, what are we trying to conserve? It must be realized that this is a devastated habitat: if the human and domestic animal populations are considered, the ratio one to the other, and the density, in the poorest parts of the Highlands, are of the same order as in parts of Africa where the country is described as semi-desert.

All the evidence shows that the Highlands were once much more fertile than they are to-day. Remains of large trees are found in desolate peat bogs; hillsides now bare and scree-covered show a brown forest soil below the accumulated debris of erosion; remains of human habitations are found associated with the remains of very large red deer at elevations of more than 2,000 ft., where now there is only cotton grass, heather and *Nardus*.

The blame for the present desolation that characterizes much of the Highlands has often been laid on a deteriorating climate, and while it seems fairly certain that the climate has got worse in the past 2,000 years—wetter and colder—this period coincides with man's first large-scale impact on Highland biological systems: the two cannot therefore be separated, but wherever deterioration has occurred, man has been present. It is therefore important to get an idea what the country was like before man interfered, if any conclusions about land-use reached are to be correct.

The Highland vegetation about 2,500 years ago consisted mainly of forest, grading into shrubby heath at 2,000–3,000 ft., which merged into arctic alpine vegetation where the hills were high enough. Tension zones would have existed between these vegetation types, and in the deteriorating climate both blanket and raised bog tended to form extensively, and these gradually killed out much forest on the higher slopes. Felling and grazing would mean retreating forests on the lower slopes. Work in another mountainous area, the English Lake District, has shown that if the nitrogen content of lake de-

posits is taken as a measure of soil fertility at the time of deposition, and the flora of that time assessed from pollen remains, then fertility fell at the same time as the forests were cleared.

Though the natural biological system has been evolved over millennia, and is best adapted to the environment, it is not practicable to re-afforest all the land that has been cleared in the past two thousand years, and a compromise must be reached.

The failure of the 1745 rising and the break up of the clan system, with the introduction of sheep to the Highlands on a large scale for the first time, resulted in rapid devastation of the forests, already depleted by earlier fellings, and the slow death of large forest areas due to the prevention of natural regeneration by grazing. Sheep have thus been abundant in the Highlands for only 200 years, and though compilation of an accurate record of sheep populations presents great difficulties, it does seem that while numbers in the Highlands have declined in the past ninety years, in the Southern Uplands they have remained steady. This decline is probably due in part to too great a drain being made on the meagre natural resources of the Highland areas: the rocks are in general metamorphic formations poor in nutrients, with more fertile sedimentary deposits only occurring in quantity in Orkney, Caithness and the Moray Firth area. Agriculture in these areas must therefore be at a lower level of productivity than in areas more favoured geologically and climatically. Recent work by the Hill Farming Research Organization has shown that one reason for the gradual decline in the fertility of sheep runs is the feeding and manuring pattern, which causes a redistribution of nutrients from one vegetation type to another; in addition there are the normal losses due to leaching and marketing sheep, which must be made good artificially. The high ratio of sheep to cattle has been cited as a cause of degradation, and as the presence of cattle helps to put right the damage due to differential manuring, the two animals are truly complementary; any relative increase in the numbers of cattle is therefore beneficial.

The problem of spread of bracken is important in the Highlands, and though clearance is expensive there appears to be little doubt that with modern methods much quite good land could be cleared economically.

A great increase in the land area under forest in the Highlands has already been achieved by private owners as well as by the Forestry Commission, and the Commission now has authority for 'social planting' in areas where it would normally be uneconomic. The soil types available for Highland afforestation are peaty podzols with iron pan, peaty gleys, iron podzols, brown forest soils and deep peat. Peaty podzols and deep peat are two difficult types available in large amounts, and on both these the problems of establishment have been solved, now that suitable ploughing equipment has been developed and the need for additional phosphate realized; additional potash will probably be required on many peat soils. Pole-stage crops at least can be raised on deep peat dominated by *Trichophorum*, and unless windthrow proves serious, plantations should be successful in many Highland areas hitherto considered unplantable. It must be remembered, however, that blanket bog is the climatic climax in many areas.

Erosion has often resulted from deforestation, long-term overgrazing and bad draining and ploughing practices; these causes of erosion can be eliminated, though much damage has already been done. If in the relatively equable climate of Scotland erosion never takes place on a tropical scale, sheet erosion still accounts for an enormous loss of top soil, though this is seldom admitted by farmers. The practice of repeated burning, especially on peaty soils, results in degradation of the flora and eventual erosion.

A number of interests and organizations are concerned with the Highlands—forestry, crofting, sheep and cattle farming, the Hydroelectric Board, fishing, shooting, and not least tourism. At present, these interests tend to go their own ways. Rural development surveys of a number of Highland areas covering more than 3,000,000 acres have recently been made by the Department of Agriculture, and in consultation with the Forestry Commission they are deciding the best division of land for each naturally demarcated area; however, there is no guarantee that owners and crofters will co-operate in these schemes.

The future of the Highlands is bound up with the future of crofting. Whether, in this age of mechanized agriculture, crofting as it is at present practised can survive is still doubtful. Unfortunately, such a haze of emotion overlies and obscures the whole subject that it is very difficult to obtain an objective view. Although where the Forestry Commission has been working on any scale the drift to the towns has been stopped, crofters are not always willing to work for the Commission, and labour may have to be brought into areas where unemployment relief was the original objective.

The integration of farming and forestry is a prerequisite of proper land use. There are, however, conflicting claims, for if lower and middle slopes of glens are planted with trees, the ratio of inbye to hill land —usually about 6·94 per cent, which is already too low—will be still further lowered. A possible solution is to use red deer as grazing animals for the high tops, treating them as a natural resource. The population of red deer is admitted to be higher than the land can reasonably carry, and as it is undesirable to re-introduce the carnivores which are the natural predators, man must control their numbers. For example, the Nature Conservancy has calculated that the herd of red deer on Rhum should be reduced by a half to two-thirds, and the same is probably true for most parts of the Highlands.

A comparison with other regions may show what is needed. In Sweden, agriculture is subsidized by forestry, and English and Lowland agriculture is effectively subsidized by industry, while in the Highlands, some of the natural resources, for example electric power and water, are exported with little or no return: the Hydroelectric Board benefits from afforestation by a reduction of the rate of silting in reservoirs, and water boards benefit from an evening-out of run-off.

Work by the Nature Conservancy, Forestry Commission Research Branch, and Hill Farming Research Organization, to mention only three bodies, is filling in many gaps in our knowledge of Highland biological systems, but the general impression the reviewer gained from this stimulating symposium was the need for more basic information, and for a more vigorous, integrated, approach to the Highland problem by all concerned.

W. O. Binns

OBITUARY

Prof. G. O. Sherrard

With the death in September last of Prof. G. O. Sherrard, affectionately known to all as 'G. O.', horticulture in general and Irish horticulture in particular has lost a most distinguished devoted scholar. He was born seventy-six years ago on the family estate near Cork City. As a boy, he had two great ambitions, to become a gardener and to teach. His father appeared to have different ideas and sent him to Westward Ho, Old United Services College and later to Woolwich Military Academy, where he received his commission.

A wish to travel prompted Sherrard to volunteer for foreign service. He was sent to Gibraltar, where he contracted Malta fever. Home on sick leave and advised to adopt an outdoor life, he naturally and fortunately chose horticulture. The soldier turned gardener now trained at Albert College, and afterwards entered the Royal College of Science, Dublin, on a scholarship, where in 1910 he obtained the A.R.C.Sc.I.

After a short period as a teacher of rural science in Co. Kildare, he was awarded a scholarship by the Development Commissioners and went to the John Innes Horticultural Institution to study genetics and plant breeding under Prof. W. Bateson. He interrupted study for active service in the First World War, reached captain's rank and was awarded the Military Cross.

From war to study again, and then back to Ireland as lecturer in horticulture in the Royal College of Science. At the foundation of the Faculty of Agriculture in 1926 he transferred to University College, Dublin, where in 1940 he became professor of horticulture—Ireland's first horticultural professor.

The Royal Horticultural Society of Ireland awarded him its Gold Medal for conspicuous service to Irish horticulture in 1941, and in 1953 the Horticultural Education Association elected him as president, the first and only Irishman to be so honoured. He was an active member of the council of the Royal Horticultural Society of Ireland and was president of that Society during 1955–58.

Prof. Sherrard was a wonderful teacher and a fine research worker, who contributed nobly to Irish prosperity. In his life-time, he witnessed a complete change in the horticultural pattern—a transition from the big estate garden to that of the suburban villa, and the remarkable development of commercial horticulture within the past quarter of a century. Quick to perceive the change, and adequate to cope with the changing requirements, he provided trained personnel for advisory, research and administrative posts. He was an inspiration and guide at a very critical period.

Although Sherrard's name as a research worker tends to be linked almost exclusively with his wonderful pioneering work on Brussels sprouts, he found time during a very busy life to publish papers dealing with the nutritional, breeding and cropping aspects of vegetables, fruits and glasshouse crops. He gave many public lectures, wrote a book on fruit and vegetable growing, contributed weekly press articles on popular gardening, and through friendly informative weekly gardening talks from Radio Eireann became the doyen of Irish broadcasting.

His outstanding work has been concerned with the breeding and improvement of commercial strains of Brussels sprouts, on which he embarked in 1924. Out of the chaos of that time he created a high order, and as monuments to his skill, patience and abiding interest he has left behind such wonderful varieties as Glasnevin Supreme, Irish Elegance and the freezer Irish Glacier. In whatever clime this crop is grown, Sherrard's name will be remembered and honoured.

Modesty and simplicity were innate characteristics of the man who put Glasnevin and Ireland before self-exaltation. An octogenarian, thinking aloud at his graveside, summed up what all of us who knew him felt in our hearts—"he was a great man and a thorough gentleman". We are the poorer for his passing, but take heart from his fine example.

E. Clarke
A. Turner

NEWS and VIEWS

General Post Office Engineering Appointments:

The Postmaster General has approved the appointment of Mr. A. H. Mumford, deputy engineer-in-chief, to be engineer-in-chief in succession to Sir Lionel H. Harris, who is retiring on January 31. Capt. C. F. Booth and Mr. D. A. Barron, both of whom are assistant engineers-in-chief, have been appointed deputy engineers-in-chief.

Sir Lionel Harris, K.B.E.

Sir Lionel Harris joined the Post Office Research Branch at Dollis Hill in 1922, having spent four years with Signals in the Australian Imperial Forces and graduated in the University of London from the City and Guilds Engineering College. He was superintending engineer of the North Midland District of the Post Office on the outbreak of war in 1939, when he left to join his Territorial Army unit. After Dunkirk he successively commanded G.H.Q. Signals, was chief signal officer, Lines of Communication; and finally for two years chief of General Eisenhower's Telecommunications Section. After the War he became for four years director of the Post Office in Scotland, then controller of research and finally (in October 1954) engineer-in-chief.

Mr. A. H. Mumford, O.B.E.

Mr. A. H. Mumford was born in London in 1903 and was educated at Bancroft's School and the University of London. He entered the Post Office in 1924 as an assistant engineer; before very long he joined the Dollis Hill Research Laboratory, where he

was particularly concerned with short- and ultra-short-wave radio systems and with multi-channel telephony and television transmission over coaxial cables. He was involved in the development of the first trans-Atlantic radio-telephone service, visiting the United States in connexion with this in 1927. He was also closely connected with work on the remote-controlled short-wave radio transmitters when the Post Office Rugby Radio Station extension was being designed. He was in charge of the Post Office Radio Branch during much of the war period. He became deputy engineer-in-chief of the Post Office in 1954. Mr. Mumford has been a vice-president of the Institution of Electrical Engineers since 1958.

Capt. C. F. Booth, O.B.E.

Capt. C. F. Booth started in the Post Office in 1923 and was for twenty-five years in the Radio Branch Laboratories of the Post Office Research Station at Dollis Hill. He is an international expert on quartz-crystal and primary-frequency standards and is part author of a comprehensive text-book on the subject "Quartz Vibrators and their Applications". He is at present leading the United Kingdom delegation to the International Telecommunications Union Radio Conference in Geneva. In 1952, Capt. Booth was appointed deputy director of the newly established External Telecommunications Union. He returned to the Engineering Department as assistant engineer-in-chief of the Post Office in 1954.

Mr. D. A. Barron

Mr. D. A. Barron was born in Bristol in 1907 and was educated at Bristol Grammar School and the University of Bristol. He entered the Post Office Engineering Department as a probationary assistant engineer in 1927 and gained his early experience in the Technical Section of the former South Western Engineering District at Bristol and in Plymouth. He was transferred to the Telephone Branch of the Engineer-in-Chief's Office in 1940 as assistant staff engineer. In 1945, he led a team which went to India to prepare plans for the transfer of the Calcutta telephone service to automatic working. On his return to Britain in 1947 he was placed in charge of a working party which examined the problems of subscriber trunk dialling which was introduced in Bristol a year ago. He was appointed assistant engineer-in-chief in 1954.

Animal Research at the Commonwealth Scientific and Industrial Research Organization : Mr. D. A. Gill

Mr. D. A. Gill, chief of the Commonwealth Scientific and Industrial Research Organization Division of Animal Health and Production, has retired after a lifetime of service to the animal industry. A graduate of the Royal College of Veterinary Surgeons, London, he joined the Organization in 1934, succeeding the late Sir Ian Clunies Ross as officer-in-charge of the McMaster Animal Health Laboratory in the grounds of the University of Sydney. He took a leading part in the practical development of methods for the control of blow-fly strike in sheep. He has been influential in improving facilities for research for the sheep industry and was closely associated with the establishment of the Organization's laboratories at Prospect and Armidale, New South Wales, and Yeerongpilly, Queensland. For some years Mr. Gill lectured on veterinary bacteriology at the University of Sydney. During the Second World War he was seconded for a time as pastoral adviser to the Department of War Organization of Industry. Instead of appointing a successor to Mr. Gill, the executive of the Commonwealth Scientific and Industrial Research Organization has decided to split the large division (which has an annual budget of £850,000) into three divisions, namely, the Division of Animal Genetics, the Division of Animal Health and the Division of Animal Physiology.

Dr. J. M. Rendel

The new chief of the Division of Animal Genetics will be Dr. J. M. Rendel, of Sydney. Dr. Rendel went to Australia in 1951 from the Institute of Animal Genetics in Edinburgh. As assistant chief of the Division of Animal Health and Production he has taken part in much of the Organization's animal breeding work, and has been particularly interested in cattle. In 1958 he made a report on the cattle industry in Papua and New Guinea for the Department of Territories.

Dr. T. S. Gregory

Dr. T. S. Gregory, of Melbourne, has been appointed chief of the Division of Animal Health, with Dr. D. F. Stewart, of Sydney, as associate chief. Dr. Gregory has been on the Organization's staff since 1926, except for a period of eight years as lecturer in bacteriology at the University of Melbourne, and during the Second World War.

Dr. I. W. McDonald

The Division of Animal Physiology will be led by Dr. I. W. McDonald. of Sydney. Dr. McDonald gained a Hawkesbury diploma in agriculture before undertaking his course in veterinary science at the University of Sydney. Before being appointed officer-in-charge of the Organization's Sheep Biology Laboratory at Prospect in 1955, he had spent several years at the Institute of Animal Physiology in Cambridge, England.

Underground Test Explosions in Britain

The Prime Minister said in the House of Commons on July 30 that H.M. Government has decided that tests involving small underground firings of conventional explosives should be carried out in the United Kingdom to obtain information on the detectability of small underground nuclear explosions. He emphasized that no radioactive materials would be used. The United Kingdom Atomic Energy Authority more recently announced that preparations were in hand for the first series of tests to be carried out towards the end of this year in a disused mineshaft in the Kit Hill area, north of Callington, Cornwall. The Atomic Energy Authority, in consultation with the Atomic Energy Division of the Geological Survey, has now located a suitable site for a further series of tests at the Greenside lead mine, Glenridding, Westmorland. This mine was about to be closed. The owners of the mine are co-operating in the arrangements and have agreed to carry out the civil engineering work involved in preparing the underground chambers in the mine for the tests. Preparations at the mine will begin shortly, and it is planned to carry out the tests in a few weeks time. The potential productive capacity of the mine

will not be impaired by the tests. The proposed series of tests will investigate the effects of firing charges at greater depths, and of greater size than is possible in the Kit Hill mine. Precautions will be taken to ensure that there will be no surface disturbance or damage. It is planned to register the results on seismic instruments at various distances from the mine. Local authorities and other interests concerned are being consulted and planning permission is being sought.

British Neutron Source Thermal Reactor

THE United Kingdom Atomic Energy Authority has signed a contract with the Hawker Siddeley Nuclear Power Co. for the purchase of a reactor to be installed at the Atomic Energy Establishment, Winfrith, Dorset, to be in operation by December 1960. Contracts for shielding, and other equipment associated with the reactor, are being placed. The reactor, which will be known as *Nestor* (Neutron Source Thermal Reactor), is based upon the Company's *Jason* reactor. Its purpose will be to provide the neutrons required for the experimental assemblies of nuclear fuels and moderators which are used to obtain data for the design of future reactor systems. To meet the requirements of the Authority, the *Jason* reactor has been modified to be capable of continuous operation at a power of up to 10 kW. and its layout has been altered so that it can drive up to five experimental assemblies simultaneously. Details of the modifications have been worked out in collaboration between the Authority and the Company. The nuclear fuel in *Nestor* is an alloy of highly enriched uranium and aluminium arranged in an annulus. Natural water is used both as coolant and moderator. The reactor is capable of operation of 10 kW. giving a thermal neutron flux of 10^{11} neutrons per cm.^{2} per sec. in the centre of the core and of up to 10^8 neutrons per cm.^{2} per sec. in typical assemblies. The thermal neutron flux can be varied by altering the reactor power-level, by using neutron shutters or by arranging the fuel loading in the annular core.

Dungeness and the Nature Conservancy

IN the light of the Government's recent decision to consent to the construction of a large nuclear power station on Dungeness, the Nature Conservancy states that the direct and indirect repercussions of the power station on the remaining area show conclusively that the future scientific interest will be too much reduced to qualify it for the status of a national nature reserve. The proposed Dungeness National Nature Reserve is accordingly abandoned and the negotiations for it will not be pursued. The Conservancy will not, however, cease to concern itself with the preservation of such assets as can still be saved. For this purpose the undeveloped area (apart from that section now compulsorily purchased by the Central Electricity Generating Board) will remain designated by the Nature Conservancy under Section 23 of the National Parks and Access to the Countryside Act, 1949, as a 'site of special scientific interest', and the Conservancy will accordingly continue to be consulted about any further development which may be proposed for any other parts of the area. The Conservancy will in particular welcome and encourage any measures which the Royal Society for the Protection of Birds may be able to take for maintaining and defending the fauna and flora and, in particular, the bird-life of the 1,234 acres of shingle which it owns between the power station site, the Lydd Army Ranges and the bungalow settlement. If Kent naturalists wish to attempt further salvage work in the remaining area of the 'site of special scientific interest' they will have the Conservancy's support. The Conservancy will continue to keep in touch with the Central Electricity Generating Board and its contractors with the view of minimizing destruction of scientific interest over the area surrounding the power station site.

Field Studies

ANNUAL reports of the Field Studies Council have recorded accounts of scientific investigations which have been made in areas surrounding the Council's field centres in Pembrokeshire, Suffolk, Surrey, Yorkshire, Salop and Devon. The growth of these centres and the increasing amount of original work which is being carried out at them have made it impossible to publish anything but a small part of the material available. The Council has now decided to issue an additional publication about new knowledge being gained in field-studies connected with archæology, botany, geography, geology and zoology. "Field Studies" will appear once yearly and, in time, it is hoped that the series of papers published for each centre will have grown into a thorough local survey. The first issue ("Field Studies", Vol. 1, No. 1 (May 1959). Copies obtainable from the Publicity Secretary, Field Studies Council, Ravensmead, Keston, Kent. 5*s*.) describes the birds, land-use, farm practice, and climate of parts of the Dale peninsula; the land snails of Flatford; the geomorphology of the Tillingbourne; stratigraphy and pollen analysis of Malham Tarn and Tarn Moss; the geography of the Alberbury Breccia; and freshwater studies in the Shropshire Union Canal.

The Young Darwin

To mark the hundredth anniversary of the publication of Darwin's "Origin of Species", a commemorative oration was delivered by Prof. C. F. A. Pantin at Christ's College, Cambridge, on September 29 ("Young Darwin and the 'Origin of Species'." By Dr. C. F. A. Pantin. Pp. 17. Cambridge: Christ's College, 1959). The address was noteworthy for a number of features. Attention was concentrated not on an old man suffering from ill health but on the pleasant, virile young man who went up to Christ's College in 1828 to prepare himself for the Church; the reasons for Darwin's subsequent ill health were also examined by Prof. Pantin, who suggested that this may have been due not to any suppressed psychological antagonisms towards his father, but to the hardships and rigours encountered during the five-year voyage on H.M.S. *Beagle*, a 10-gun brig of only 242 tons. Darwin's contributions to knowledge in many branches of natural history include references to the origins of coral seas. There are also some valuable gleanings of the way in which "The Origin of Species" emerged.

The London Mathematical Society

THE annual general meeting of the London Mathematical Society was held on November 19 at Burlington House. The De Morgan Medal was presented to Sir William Hodge, physical secretary of the Royal Society and Master of Pembroke College, Cambridge. In presenting the Medal, the president (Prof. H.

Davenport) paid tribute to Sir William's distinguished work in algebraic and differential geometry. Prof. Davenport briefly reviewed the Society's affairs, and announced that a loan granted by the Nuffield Foundation had enabled the Society to begin reprinting those of its publications which are out of print. He also announced that the Oxford University Press had agreed to publish the Collected Papers of G. H. Hardy, with the assistance of an editorial board appointed by the Society. Prof. H. Heilbronn (University of Bristol) was elected president for the ensuing session. The retiring president delivered his presidential address on "Some Recent Progress in the Analytic Theory of Numbers".

Model Marine Engines

Liverpool Public Museums have recently placed on exhibition a series of working models of steam reciprocating marine engines. They cover the period 1837–1937 and start with the side-lever paddle engine of 1837. Then comes a set of oscillating cylinder paddle engines of the 1840's. Also in the paddle engines section is a set of diagonal compound machinery of about 1890. Screw propulsion came into general use during the 1840's and '50's, and the earliest model is of the horizontal return connecting-rod type. All the remaining screw engines are inverted direct-acting. The models are displayed working, and the side-lever and oscillating cylinder engines run at ten and twelve revolutions a minute, which is their correct working speed. The other models run at forty revolutions a minute, which is well below the average speed of their real prototypes. Running at this low speed is necessary, however, to preserve the bearings of the models, for they have been acquired for posterity as well as the present generation. The models are fully described in a paper by Mr. E. W. Paget-Tomlinson in the October issue of the *Museums Journal*.

Replicas of Workshops

The *Museums Journal* of September includes an account by Mr. Keith Dunham of the workshops which have been reconstructed in the Museum of Science and Industry at Birmingham. The city has always had a reputation for a multiplicity of trades, all of which have grown from the skill in iron-working possessed by the inhabitants since early times. The building erected in the mid-nineteenth century in which this Museum is now housed was formerly the premises of a firm of silversmiths and electroplaters, and thus gives an excellent background for reconstruction of early workshops. The trades chosen were those of the brass-turner, the action filer (gunmaker), the nailer, the jeweller and the cooper. The workshops of a silversmith and brass-founder were already in existence. Details of the display are given in the paper quoted.

Phycology in Britain

The recent issue of the *British Phycological Bulletin* (No. 7, July 1959) contains the presidential address, "Buoyancy in Relation to the Ecology of the Freshwater Phytoplankton", presented to the Society by Dr. J. W. G. Lund, together with abstracts of the other papers read at the London meeting in January 1959. There are also the first part of a list of the chromosome numbers in the Chlorophyta, notes on the algal herbaria of Hudson and Lightfoot, a list of marine algae collected in the course of field excursions organized during the Third International Seaweed Symposium held in Galway in July 1958, reviews of recent phycological literature and a number of shorter articles.

Prize for Man-powered Aircraft Flight

The Royal Aeronautical Society announces that a prize of £5,000 has been offered by Mr. Henry Kremer for the first successful flight of a man-powered aircraft, designed, built and flown within the British Commonwealth under conditions to be laid down by the Royal Aeronautical Society. The Royal Aero Club is to be invited to act as official observers. The prize is offered personally by Mr. Kremer and not by any of the companies with which he is associated. Mr. Kremer has been on the production side of industry all his life, and before the War pioneered the resin-bonded composition board industry. After the War he began to take an interest in plastics, and in 1950 he formed Microcell, Ltd., which has been extended to include an aircraft engineering division and an electronics division, as well as the plastics division. Since 1950 two other companies have expanded under his chairmanship, namely, Glass Yarns and Deeside Fabrics, Ltd., producing glass fibre, and Artrite Resins, Ltd. Microcell, Ltd., has since merged with BTR Industries.

Postdoctoral Awards : U.S. National Science Foundation

Postdoctoral fellowships are offered by the U.S. National Science Foundation to citizens of the United States with special aptitude for advanced training and who hold the doctoral degree or have the equivalent in training or experience. Fellowships will be awarded in the mathematical, physical, medical, biological, engineering and other science fields, including anthropology, psychology (other than clinical), geography, certain interdisciplinary fields and selected social science fields. A grant of 4,500 dollars a year will be awarded to successful applicants under the postdoctoral fellowship programme. Dependency allowances will be made to married Fellows. Fellows may engage in study and/or research at appropriate non-profit American or non-profit foreign institutions. A limited allowance to aid in defraying a Fellow's cost of travel will also be available. Further information can be obtained from the Fellowship Office, National Academy of Sciences—National Research Council, 2101 Constitution Avenue, N.W. Washington 25, D.C. All applications must be made by December 22.

"Animal Clocks"

Referring to the report of his paper at the York meeting of the British Association in the symposium on "Animal Clocks", published in *Nature* of September 12, p. 763, Dr. Malcolm B. Wilkins states that the last two sentences should read as follows: "The period of the rhythm is slightly sensitive to temperature. Ball and Dyke have shown that the growth-rate rhythm in *Avena* coleoptiles is inhibited in nitrogen, and Bünning has shown that the period of the rhythm of leaf movement of *Phaseolus multiflorus* is increased by the mitotic poisons colchicine and phenylurethane."

University News : Leeds

The following grants are announced: £4,310 for the period of one year from the National Coal Board.

Scientific Department, for six research projects in mining; £3,860 from the Central Electricity Generating Board for research on the utilization of pulverized fuel ash in the Department of Agriculture.

Dr. A. F. Thomas has been appointed lecturer in the Department of Organic Chemistry.

London

THE following appointments are announced: Mr. P. R. Bell, of University College, to the University readership in botany tenable at that College; Mr. A. V. Bradshaw, of the Imperial College of Science and Technology, to the University readership in extraction metallurgy tenable at that College.

The title of professor of electrical engineering in the University of London has been conferred on Dr. A. H. M. Arnold, in respect of his post at King's College.

Oxford

RESEARCH grants are announced as follow: from the Department of Scientific and Industrial Research a grant not exceeding £35,900 for the period July 1, 1959, to July 31, 1962, for research on high-energy phenomena using photographic plates, being carried out under the direction of D. H. Wilkinson, professor of experimental physics; from the United Kingdom Atomic Energy Authority a grant not exceeding £8,500 for the period October 1, 1959, to September 30, 1962, for a study of polarized nuclear systems using resonance techniques, to be carried out in the Clarendon Laboratory under the direction of Dr. F. N. H. Robinson; and from the Ministry of Aviation a grant not exceeding £1,395 for the year beginning October 1, 1959, for research on the mechanism of operation of the cryotron, being carried out in the Clarendon Laboratory under the direction of Dr. K. A. G. Mendelssohn, reader in physics.

Sheffield

IT is announced that the Strength of Materials Laboratory in the Department of Mechanical Engineering is to be named the F. C. Lea Laboratory, in honour of the late Prof. F. C. Lea, who was professor of mechanical engineering during 1923–36 and whose researches carried out in Sheffield made important contributions to knowledge in this field.

Announcements

MR. BRYAN HARRIS, a graduate in metallurgy from the University of Birmingham, has been appointed to a Tube Investments Research Fellowship for work in the physics and chemistry of solids, at the Cavendish Laboratory, Cambridge.

MR. G. G. GOURIET is to give the Christmas Holiday Lecture of the Institution of Electrical Engineers on December 30, at 3 p.m., and repeat it at the same time on the following afternoon. He will speak on "Colour Television". Tickets, which are free, must be obtained from the Secretary, Institution of Electrical Engineers, Savoy Place, London, W.C.2.

THE Science Museum, South Kensington, London, S.W.7, has arranged the following Christmas lectures for boys and girls: December 22 and 23, "From Man Power to Atomic Power", by Mr. Victor Wall; December 28 and 29, "From Tinfoil to Tape: the History of the Gramophone", by Mr. John Cain; December 30 and 31, "The Story of Flight", by Mr. Victor Wall; January 1, "Science and Magic", by Mr. John Cain.

THE Zoological Society of London has arranged the following Christmas Lectures for children: December 29, "Looking for Animals in East Africa" by Mr. E. H. Tong; December 31, "British Carnivores" by Mr. R. F. S. Creed; January 4, "Animals in Hiding" by Mr. D. G. Lambert. The lecture hour is 3 p.m. and admission is free by ticket obtainable on application to the Education Department, Zoological Society, Regent's Park, London, N.W.1; only three tickets can be supplied to a Fellow for each lecture.

THE seventh Sir Thomas Middleton Memorial Lecture will be given in the Auditorium, Wellcome Building, 183 Euston Road, London, N.W.1, on December 8 by Dr. E. B. Worthington (deputy director-general (scientific), Nature Conservancy), entitled "Nature and Agriculture". Further information can be obtained from Miss D. F. Bray, Sir Thomas Middleton Memorial Trust, 3 Dean's Yard, London, S.W.1.

A TWO-DAY symposium has been arranged by the Linnean Society during December 17–18, entitled "Quaternary Ecology". The meeting will be held at Burlington House, Piccadilly, W.1. Further information can be obtained from the General Secretary, Linnean Society of London, Burlington House, London, W.1.

THE British Society of Rheology will hold a meeting in Manchester during April 7–8, and will include sessions on lubrication, disperse systems, and the methods and concepts of rheology; and a choice of visits to British Rayon Research Association, the Shirley Institute, Jodrell Bank and the Fluid Motion Laboratory of the University of Manchester. Full details can be obtained from the Hon. Secretary of the Society, 8 The Broadway, Pontypool, Mon.

THE Electronics and Communications Section of the Institution of Electrical Engineers, in association with the International Federation for Medical Electronics, is organizing the third International Conference on Medical Electronics which will be held at Olympia, London, during July 21–27, 1960. Further information can be obtained from the Secretary, Institution of Electrical Engineers, Savoy Place, London, W.C.2.

SUNVIC CONTROLS, LTD., is to combine with the Instrument and Meter, Scientific Apparatus and X-Ray Departments of Metropolitan-Vickers Electrical Co., Ltd., to form the Instrumentation Division of Associated Electrical Industries, Ltd.

IT is announced by Birlec, Ltd., that the name of the company is to be changed to A.E.I.–Birlec, Ltd. The change coincides with the final reorganization of the structure of the parent company, Associated Electrical Industries, Ltd., into several product divisions. The internal organization of Birlec, Ltd., will not be affected. The company will continue to operate as in the past for the manufacture of heat-treatment furnaces, gas plants and adsorption dryers. The name of its associate company, Birlec-Efco (Melting), Ltd., will not be changed.

ERRATA. In the communication entitled "Nature of the Olefines produced by Apples" in *Nature* of September 26, p. 996, par. 2, line 15, for "1-hexane" read "1-hexene"; par. 3, line 4, for "necessary" read "unnecessary".

BRITISH IRON AND STEEL RESEARCH ASSOCIATION

LONDON GROUP LABORATORIES OPEN DAYS

THE British Iron and Steel Research Association recently held open days at its Battersea premises to show its members something of the current and continuing work of the London Group laboratories. Of the five Divisions and three Departments of the Association, the London Group comprises the Plant Engineering and Energy Division, part of the Iron-making Division, and the Physics, Chemistry and Operational Research Departments.

Notwithstanding the wide spread of interests represented, the displays clearly reflected the interest of the steel industry in automation and allied subjects. Not only has the Physics Department recently formed a new Section to deal specifically with the subject of automation, but also the Electrical Engineering Section of the Plant Engineering Division has pioneered several developments which, in effect, apply 'automation' in the handling of information.

Some years ago this Section developed a 'performance recorder' which recorded and stored production data, and processed it so that periodical tabulated totals would be available in printed form for office use. Equipment of this kind has since been installed in several British steel-works and other factories, and a development of the basic idea was designed and supplied for a big Russian contract for the erection and equipment of a giant tyre factory in the Soviet Union by a British consortium.

The Electrical Section of the Association has now completed a further development—the 'Tallimarker' system for conveying essential information in a steel-works. Starting from the premise that efficient production depends on accurate, easily accessible information, this automatic system represents a great advance on the traditional pattern of circulating data by paper chits or word of mouth. It consists basically of three essential units: 'push-button' desks, and display screens at key points along the production line, and a central 'memory'. As applied in a steel-works rolling mill, for example, the operators at the soaking pit, primary mill, primary shears, section mill and saw-bench, would all have press-button stations into which information is fed, and all stations after the soaking pit would have a display screen showing the previous history of the product as it is progressively reduced from the original ingot. As the ingot or subsequent bloom passes down the line, the operator at each point reads off the facts on his display screen, and feeds in more information as he completes his operation, for the following stations. When a section is being cut at the final saw, the 'Tallimarker' store holds the complete history of the section. Details are relayed to the mill office and recorded in the most convenient form; for example, on a teleprinter record, punched card or magnetic tape. The basic system can, of course, be adapted for use in any production line; in construction it is reliable, robust and accurate, reduces clerical work, and saves time and money.

The new Automation Section of the Physics Department is studying several possible applications of automation to steel-works production methods. Among these is a scheme for the automatic inspection and treatment of rolled billets, which often have surface defects which must be made good. The new plan is to dip the billet in a fluid which fluoresces under ultra-violet light, and which penetrates the flaws. The billet will then be washed and passed to an inspection chamber, where the flaws show up under ultra-violet lamps and can be detected by photocells. The photocells can be linked electrically with finishing tools such as grinders or de-seaming torches for automatic treatment. Billets without flaws would be diverted and stored, later rejoining the line in their original order.

The increasing use by manufacturers of steel sheet and strip has led to a growing pre-occupation with methods of quality control. Automatic gauge control was developed by the Association some years ago, and has been widely adopted in industry. On show at Battersea was a proposed new method of measuring small reductions in strip thickness, which can be integrated with automatic correcting equipment. The system is based on the fact that the reduced strip leaves the rollers faster than it enters them—the greater the reduction, the greater the change in speed. Therefore, by measuring the change in speed, the reduction can be calculated. It is proposed to do this by comparing identical signals obtained by photo-electric measurement of reflectivity at two points 'down the line'. The time-lag between the signals gives the time the sheet or strip takes to travel the distance between the points of measurement. One of these instruments before and one after rolling will give the change in speed, and therefore the reduction in thickness. This 'non-contact' method avoids any complications which would arise from the use of contact rollers or similar devices.

Because automatic control demands as an essential prerequisite exact information, instrumentation may be said to be the first step towards automation. An interesting new instrument is the 'bar section meter' displayed by the Physics Department. It has been designed to measure simultaneously four diameters of a hot solid bar at 45° intervals while it is moving at a high speed. This information is valuable not only for checking that the section is being rolled to the specified size, but also for early detection of such defects as finning.

The water-cooled viewing head forms images from the opposite edges of the bar across each diameter to be measured. These images are placed side by side in a single field of view, and are magnified and transposed so that the edges point towards each other. The instrument thus shows four parallel bands of light representing the four diameters measured. Each nominal diameter of the bar is set on the instrument by a micrometer adjustment; if this corresponds to the diameter being viewed, then the appropriate band is continuous across the field of view (that is, the fact that the edge images have been brought to coincidence in any band indicates that the diameter

is as set). If there is a gap in the middle of the band, the diameter of the bar is less than the scale reading, while a bright central zone caused by the images overlapping indicates that the diameter is greater. First experiments show that deviations in diameter of about 0·005 in. can be detected.

Leaving the theme of automation, an interesting sidelight on the technique of research was provided by the Ironmaking Division. Although the blast furnace is a remarkably efficient vessel for the large-scale exercise in physical chemistry which ironmaking represents, there is still much to be learned about the course of the reduction process. There are severe limits on what can be deduced from experimental work on a blast furnace, and the Division therefore developed laboratory apparatus to simulate the reactions taking place in the blast furnace. The *Scice* apparatus (stationary charge in controlled environment) metaphorically turns the blast furnace 'inside-out'. In the blast furnace the charge moves down the stack, encountering changing conditions and undergoing various reactions; in *Scice* the charge is held stationary while gas of varying compositions and temperatures corresponding to these changing conditions is passed through it and reacts with it. By controlling the conditions continuously, the ironmaking process can be reproduced and its reactions studied with relative ease.

The practicability of this approach was originally proved in the laboratory by experiments using a charge container of three inches diameter. A much larger and more elaborate version has now been installed at the Battersea Laboratories. It incorporates a gas producer, gas absorbers, gas booster, electric heater for the gas, a reaction chamber 20 in. in diameter by 24 in. high, dust cyclone, and a gas cooler. Instrumentation has been provided for flow-rates, temperatures, pressures and gas compositions, with provision for easy control of the variables. This scaled-up version of the original bench conception provides a unique tool with which to solve some of the remaining unknowns about blast furnace operation.

Also housed at Battersea is the Association's Operational Research Department, which now consists of three Sections: Computer Applications, Operational Investigations and Human Factors.

The first of these is primarily concerned with helping the steel industry to make use of the electronic computer. This involves the use of the computer not only as a research tool but also as an administrative and/or production aid. Using the Ferranti *Pegasus* computer installed at Battersea in 1958, the Section has carried out extensive studies of the feasibility and economics of using computers for such familiar problems as production scheduling, stock control, wage calculation, plant and process control, and allocation of materials, and already much valuable data have been collected.

The Operational Investigations Section is operating a service—at present confined to the Association's member firms—whereby the Department's experience and facilities can be used to supplement managerial experience with forecasts of the effects of alternative policies. A typical problem, involving a decision on stock control methods, was illustrated by displays. The example taken was a company keeping large stocks of billets to ensure against production hold-ups. This involved problems of locked-up capital and periodic rises in storage and transport costs.

The first step was to analyse existing order and stock control methods. From this, and from quantitative records, the main characteristics of production and raw material delivery over a long period were mapped out, and the effects of different control methods simulated on paper and carefully studied.

Eventually, a set of 'ordering rules' was drawn up which in practice proved so successful as to release £100,000 of working capital by reducing the average stock-level, and a further saving of some £6,000 annually by reduced labour and transport costs.

The Human Factors Section of the Department is concerned with such things as accident prevention, protection against heat and injury, and the broad subject of ergonomics, or 'fitting the machine to the man'. In this latter field, displays showed many advances in control equipment design, aimed at reducing operator fatigue, eliminating strain and increasing efficiency. An experimental control point was on display incorporating variable hand (and foot) controls, which has proved the genesis of a series of improvements already being adopted in production practice.

It is, of course, impossible to give other than an arbitrary selection of the features of the display which, in any event, was representative of a part only (about one-third) of the whole programme of the British Iron and Steel Research Association. This account, however, provides an indication of some types of problem being tackled and of the methods of approach.

D. U. Hunt

COMPUTER LABORATORY AT THE UNIVERSITY OF LIVERPOOL

THE University of Liverpool has had a Mathematical Laboratory for more than twenty-five years—it was established by Prof. L. Rosenhead in 1934 shortly after he became head of the Department of Applied Mathematics. The primary object of the Laboratory was always to train students in the honours school of mathematics and research students in other schools in the basic methods of numerical analysis applied to all branches of science.

The Laboratory has always been well equipped with accounting machines as well as desk calculating machines. An important feature of the teaching in the Laboratory has been the insistence on the fullest possible exploitation of all types of calculating aids, and much effort has been spent on not only investigating the possible uses of machines but also on developing methods suitable for them.

The spectacular post-war growth in the demands for numerical analysis has led to very greatly increased demands being made on the Laboratory; the problems received for solution have been growing more numerous, more complex and greater in scope. At first, the need was met in part by making use of punched card accounting machinery, and later by

using the facilities of electronic computing establishments elsewhere—particularly at the University of Manchester, the generosity of which in this respect has been greatly appreciated by several other northern universities as well as Liverpool. More than four years ago, however, it was already clear that the University of Liverpool should be equipped with its own computer, and detailed plans were made for the expansion of the Laboratory. It was therefore with very keen disappointment that the University learned two years ago that money could not be provided at that time by the University Grants Committee for the provision of a computer.

A survey made in the University had established the fact that the lack of an adequate computing service was causing research in some departments to be retarded seriously—particularly in the fields of nuclear physics and oceanography. In view of this situation, the University Council decided that a computer must somehow be provided and an appeal was launched among local business concerns and friends of the University for donations towards the cost of purchasing a machine. The response was generous—particularly as the appeal was made within a year of the major appeal to finance the general building development of the University—and as a result the University was able to place an order with the English Electric Co., Ltd., for a *Deuce*, the remaining balance of the cost being advanced by the University itself. At the same time the staff of the Laboratory was increased and its name changed to the Computer Laboratory, Mr. Andrew Young being appointed as its first director.

The machine was installed and came into operation last July. It is a 64-column *Deuce*, which is at present equipped with punched card input and output mechanisms. A comprehensive range of punched card ancillary equipment—card punches, sorter, reproducer and tabulator—has also been installed; a card-operated typewriter is due for early delivery. In addition, paper tape input and output devices and the necessary tape editing ancillary equipment have been ordered, but have not yet been delivered.

The Computer Laboratory was officially inaugurated at an open day held on October 28, when the University entertained donors to the appeal fund and members of Council and Senate. The guests were welcomed by the Vice-Chancellor, Sir James Mountford, and were shown over the Laboratory by the staff; the Director gave an account of the work being done in the Laboratory.

It is already clear that the computer is meeting a very real need. Extensive use of the machine is being made, as expected, by the Departments of Applied Mathematics, Physics—mainly analysing results of experiments made on the University's large synchrocyclotron—and Oceanography as well as several departments in the Faculty of Engineering, including the new Department of Building Science.

What has surprised—and gratified—the University, however, is the unexpectedly large use of the machine by departments which had not hitherto been expected to use it extensively, if at all. Some of the most frequent users are members of a group of physiologists working on a problem of liver function. Members of the Department of Psychology and of the Medical Research Council are now analysing the results of gerontological studies with the computer, which, because of its ability to cope with the heavy arithmetic involved in factor analysis, has enabled them to extend their work. In addition, educational, economic and social scientific surveys have been helped by the new computing facilities available.

Training in programming and using the computer and its ancillary equipment is given regularly to members of the staff of the University, research students, and to outside users, a number of whom are making use of the machine on a basis of time-hire.

It is now a commonplace occurrence that the installation of a computer leads to an expansion in the scope of the work undertaken in an institution, and it is already abundantly clear that in this respect the installation in Liverpool will prove to be no exception. The University is proud of its new project, and is confident that the decision to embark on it will be fully justified.

BRITISH ELECTRICAL AND ALLIED INDUSTRIES RESEARCH ASSOCIATION

A NEW format has been adopted for the 1958 annual report of the Electrical Research Association (Thirty-eighth Annual Report for the year ended 31st December 1958. Pp. 103. Leatherhead: Electrical Research Association, 1959). The report, which is attractively reproduced, departs from previous practice in having some fifteen illustrations, mostly photographs relating to research projects. One diagrammatic illustration of special interest shows the proportions of the income of the Association coming from various sources and the distribution of expenditure among the main fields of research, in administration and in services to members.

The total income of the Association for the year was just over £458,000, subscriptions from members totalling some £213,000, income from directly sponsored researches earning nearly £113,000, and the grant from the Department of Scientific and Industrial Research contributing £100,000. From the chart showing the distribution of expenditure it appears that some 9 per cent is used in administration. A slightly greater amount represents services to members which, in addition to general technical liaison, cover the answering of requests for advice and information and the provision of abstracts. Research expenditure appears under seven headings, divided as percentages of the total expenditure, roughly, as follows:

Properties of materials	19	Applications of electricity	12
System problems	14	Transmission	6
Control and transformation	10	Specialized plant	10
Miscellaneous researches	9		

On the basis of expenditure, properties of materials thus represents, by a substantial margin, the biggest research effort of the Association, and it is evident from the report that this effort, which is devoted largely to insulation, has contributed both to funda-

mental knowledge and to technological progress. Much of the Association's technological work in this field has contributed to the drafting of British Standard specifications and similarly, at the international level, to the recommendations of such bodies as the International Electrotechnical Commission. During the past year the Electrical Research Association's recommendations relating to electrode systems for electric strength tests and for methods of measurement of permittivity and loss angle have been so adopted.

Work conducted at the National Physical Laboratory on the effects of electron and neutron irradiation of polythene is reported. It has been shown that the loss tangent of the material is little affected by irradiation dosages up to 10 Mrads, but thereafter increases due to oxidation.

Among the investigations of a fundamental character concerning the phenomena of instability in dielectrics, one may be mentioned dealing with the formation of gas in impregnated insulation. This has established the relation between moisture content and the stress required to initiate gas production. It has been possible to observe the slow growth of breakdown channels in a very viscous liquid and this forms the subject of one of the illustrations in the report.

A reliable technique has been developed for measuring the electric strength of liquefied gases, it having been established that the state of the electrode surfaces and particularly the degree of oxidation have a marked influence on the result. The parts played by anode and cathode have been separated by using electrodes in which the cathode and anode surface were different.

The influence of impurities on the magnetic properties of silicon–iron transformer sheet has been under investigation at the laboratories of the British Iron and Steel Research Association. It has been shown that, with respect to hysteresis loss, nitrogen is much more harmful than carbon, and two methods of minimizing the ill-effects of nitrogen are being examined. One method involves applying a thin coating of aluminium to laminations before annealing while the other utilizes a small admixture of titanium.

Theoretical work on deviations from Ohm's law in semiconductors has predicted that in sufficiently strong fields mobility should decrease with the 0·8th power of the field strength. This prediction has been confirmed by measurements made at Purdue University.

Circuit breaking and associated fundamental studies in arc physics continue to constitute an important part of the research programme. The temperature within the conducting core of the arc and, in particular, the formation of successive discrete cores as the current increases, have been studied in terms of dissociation and ionization, and it has been shown that the pinch pressures occurring at the electrodes outweigh the effects of natural convection and may provide an explanation of the phenomena of metal transfer in welding arcs.

Work on the creep properties of steel continues and is being extended. The first test-results have been obtained on the bursting of thick-walled pipes under creep conditions. A new laboratory to meet the needs of the augmented programme of work on creep testing is to be built at Leatherhead.

A result of considerable industrial significance has been obtained in the course of the programme of work on flame proofness and intrinsic safety. A re-evaluation of the maximum safe-gap for hydrogen made with improved research technique has shown the safe gap to be appreciably greater than was previously accepted. The value has been established as 11 mils. This opens up the possibility of designing flame-proof apparatus for use in hydrogen and some of the other most explosive gases.

The rural electrification work of the Association, which is concerned very largely with the application of electricity to agriculture and to horticulture, has, in the past, embodied a considerable programme of research on plant environment. This continues but it is now supplemented by a parallel programme on animal environment.

In a foreword to the report the president, Sir Josiah Eccles, points out that we are entering an era in which the scale and complexity of equipment and of automatic control systems will be such as to render capital charges a dominant factor in industrial costs. There will thus be an increasing premium on ingenuity and skill in producing more effective and cheaper equipment which, in effect, means an increasing emphasis on research. Bearing in mind that the vast majority of manufacturing firms in Great Britain employ less than 500 people and that many problems can be better tackled co-operatively than in specialized laboratories, it is evident that research associations have an important part to play in the effort to develop to the full the nation's scientific potential. In furthering this development the greatest responsibility falls, almost certainly, to the electrical industry.

J. GREIG

FLOW PROPERTIES OF BIOLOGICAL SYSTEMS

A MEETING, under the joint auspices of the Faraday Society (Colloid and Biophysics Committee) and the British Society of Rheology, was held during September 23 and 24 in the School of Physiology of the University of Oxford. On the first day, the flow properties of various biological systems were discussed, while the second day was devoted entirely to blood and blood vessels. The attendance of more than seventy included a number of overseas guests.

The Conference was opened by Prof. P. R. Allison, Nuffield professor of surgery, who welcomed the meeting to Oxford and spoke of the increasing importance of rheology in medicine and surgery. A general introductory lecture, under the chairmanship of Prof. M. Reiner (Haifa, Israel), was given by Dr. A. S. Lodge. Many of his audience were not rheologists and, for their benefit, Dr. Lodge outlined the scope and methodology of this branch of physics.

Several papers were read which concerned uterine cervical mucus and the connective tissue of the cervix. Dr. F. K. Beller (Tübingen, W. Germany) described

the rheological changes which take place in human mucus during the menstrual cycle and following certain hormone treatments, and Dr. R. A. Gibbons and Mr. F. A. Glover (National Institute for Research in Dairying, Shinfield) discussed measurements of flow birefringence and the information which these might give concerning the size and shape of mucoid molecules. Drs. M. L. R. and D. R. Harkness (University College, London) were concerned with the very marked changes which must take place in the lower end of the uterus and in the cervix to allow of the passage of the fœtus at parturition. The concentration of collagen drops from 10 per cent (wet basis) in the non-pregnant animal to about a third of this amount during pregnancy.

Drs. J. C. White and P. Elmes (London Postgraduate Medical School) described viscosity, intrinsic viscosity and thixotropy measurements on dilute bronchial mucoid sputum. A special apparatus was designed to measure the yield-values of undiluted samples, which may be related to the force required to initiate movement of the mucus in the smaller respiratory passages. Dr. H. J. Rogers discussed hyaluronidase and hyaluronic acid.

Living skeletal muscle has remarkable rheological properties, contracting rapidly and completely from extensions of up to 130 per cent, but this extreme elasticity is quickly lost *post mortem*. J. R. Bendall (Low Temperature Research Station, Cambridge) spoke of the form of the deformation-time curves and the biochemical processes underlying the changes which take place in them. Also concerned with muscle, Dr. J. J. Connell (Torry Research Station, Aberdeen) described measurements of toughness and firmness of fish and of fish pastes and jellies as related to subjective assessments.

Prof. L. V. Heilbrunn (Philadelphia) discussed methods for measuring the rheological properties of protoplasm, and stressed the differences between these and the behaviour of purified proteins. Prof. J. A. V. Butler and Dr. A. B. Robins (Chester Beatty Research Institute, London) described simple viscometric measurements (using concentric cylinders) for the study of nucleic acid solutions; and the significance of intrinsic viscosities and of viscous anomalies such as dependence of viscosity on shear rate.

Dr. G. W. Scott Blair (National Institute for Research in Dairying, Shinfield) read two papers, the first of which dealt with the rather complex properties of milk coagulated by rennet which, at best, requires four parameters for its specification ('Burgers body'). On the second day, he described similar experiments on coagulated blood, which, surprisingly enough, except in the earliest stages, requires only two parameters ('Maxwell body').

The symposium on blood was introduced by Dr. L. W. Bayliss (University College, London) under the chairmanship of Prof. I. de Burgh Daly. Dr. Bayliss outlined studies which have been made on viscous anomalies in blood flowing through artificial capillaries, emphasizing the importance of the relatively easier flow which is found in the narrower tubes and which reflects axial movement of corpuscles. Dr. R. L. Whitmore (Nottingham) gave a theoretical treatment of these differential flow velocities of plasma and corpuscles in the living body. By means of radioactive tracers, changes in concentration due to radial movements can be followed. He discussed turbulent conditions, which also formed the theme of a paper by Dr. W. E. Stehbens (Sydney, Australia and Oxford), who pointed out that, especially in the neighbourhood of bifurcations, turbulence takes place at quite low Reynolds' numbers. Nor must the pulsatile nature of the flow be forgotten. Streamline conditions are not as general in the body as used to be supposed. Dr. D. A. McDonald also discussed the hydrodynamics of pulsatile flow, basing his treatment on the theoretical work of the late Dr. J. R. Womersley and of Dr. M. G. Taylor.

Prof. A. L. Copley (New York and London) discussed the nature of the flow-curve of blood in glass capillaries which has recently been shown to follow Casson's equation (see G. W. Scott Blair, Nature, 183, 613; 1959). When the surface of the capillary is coated with fibrin, the apparent viscosity falls and the differential flow velocities appear to be accentuated. Moving 'indexes' of blood in sloping capillaries also adhere less to fibrin than to glass surfaces. In the living blood vessel, an endo-endothelial fibrin film may constitute a major factor in helping circulation. He also presented work on native blood and plasma and the effects of anticoagulants.

Prof. S. Baez (New York) described pressure effects in living microscopic vessels and pointed out the important part which the tone of the vasculature plays, not only in hæmodynamics but also in those studies now often referred to as 'hæmorheology'. Dr. H. Hartert (Heidelberg, Western Germany) discussed the rheology of his well-known technique for following the process of blood coagulation by measuring the progressive increase in shear modulus ('thromboelastography') and also described his more recent invention, the 'retractograph', which follows the process of retraction, claimed to start immediately at the clotting point and to increase in parallel with increase in the shear modulus. Dr. H. H. Pfeiffer (Bremen, Western Germany) described his measurements of rheodichroism, a phenomenon which throws much light on the molecular nature and characteristics of the so-called 'leptones' which are responsible for differential light absorption in flow. Prof. A. Katchalsky (Rehovoth, Israel) and his colleagues discussed recent experiments on osmotic effects in individual erythrocytes. These behave rather like football bladders, in that they are very readily deformed up to a certain degree of swelling, after which they offer enough resistance for the pressure quickly to exceed their apparent strength, a fact which may help to account for the comparative ease with which red cells appear to pass along very narrow vessels.

In the afternoon of the second day of the meeting, there was an exhibition of apparatus. Many of the instruments associated with the papers which had been read were on view. The demonstrations were introduced by a lecture by Prof. K. Weissenberg in which he showed many strange rheological phenomena which, in less dramatic form, are likely to make their appearance in biological systems. His own 'rheogoniometer' was among the exhibits.

In the evening, films were shown which developed further the themes already described by Dr. D. L. McDonald and his colleagues, by Prof. S. Baez and by Prof. A. L. Copley, Dr. G. W. Scott Blair and their colleagues. Finally, Prof. M. H. Knisely (Charleston) showed a film to illustrate his work on 'sludged blood'.

Much of the success of the meeting was due to the excellent local organization, in the hands of Dr. R. G. Macfarlane, who also acted as honorary treasurer. The proceedings of the Conference will be published in book form early in the New Year under the joint editorship of Prof. A. L. Copley and Dr. G. Stainsby.

G. W. Scott Blair

BRITISH COLONIAL TERRITORIES

"THE Colonial Territories, 1958–59"* provides the usual convenient compendium of information to such further publications as "Colonial Research" dealing with the British Commonwealth and itself includes a 24-page chapter dealing with "Research and Surveys", to which grants totalling £800,824 were made from Colonial Development and Welfare Funds for a further 134 research schemes. The recommendations of the meeting at Lagos in January 1958 for the future organization of research in West Africa have now been accepted by the Governments of Ghana, Gambia, Nigeria and Sierra Leone, and the West African Research Office came formally into being on April 1, 1959. The Government of Ghana has now abandoned its intention of taking over the West African Cocoa Research Institute, which will accordingly continue as a fully inter-territorial research body. The Commission for Technical Co-operation in Africa South of the Sahara has decided to transfer the headquarters of the joint secretariat from London to Lagos.

Approved technical assistance projects in the British-dependent territories in 1959 under the United Nations Expanded Programme of Technical Assistance (to which the United Kingdom Government at present contributes the sterling equivalent of 2,240,000 dollars a year) amounted to 1,062,750 dollars. Expenditure from Colonial Development and Welfare Funds was £17·8 million and the Vote for Colonial Services included £24,223,000 as financial aid to Colonial Governments. Exports (excluding Northern Rhodesia and Nyasaland) fell by £46 million and imports by nearly £80 million, including about £35 million in Hong Kong and Singapore and nearly £20 million in East Africa. Capital formation in 1958 remained at about the same value as in 1957.

Among some other points which may be mentioned are the rapid expansion of educational facilities, although the provision of new school places and additional teachers remains the over-riding problem in most territories. In October 1958, the two universities and three university colleges in Central Africa had 3,783 students, compared with 3,400 the previous year, and there were 1,615 students at the University of Malaya, which serves Singapore as well as the independent Federation of Malaya. Capital sums provided under the Colonial Development and Welfare Act for university institutions are now almost completely committed, and a main financial problem is now to provide the required increase in accommodation for residence and teaching. The Singapore Polytechnic opened in November 1958 with 385 full-time, 506 day-release and 2,005 evening students, and a second new technical college in Jamaica opened in March 1958 with 60 students. In Jamaica, 11,000 extra primary places were provided out of the 16,000 required, and in Uganda the number of African pupils in aided secondary schools has risen from 5,517 in 1951 to 17,306 in 1958.

Attachments of American teachers to institutions in British Colonial territories continued under the Fulbright Agreement, and the Overseas Visual Aids Centre was officially opened in London in February. Departments of health generally were able to meet increasing demands on all services while maintaining a fair balance between curative and preventive work. Mass campaigns and surveys or pilot projects preceding them have been used extensively to combat endemic diseases, but although the incidence of malaria and yaws has been dramatically reduced in this way, tuberculosis remains a serious problem. Progressive improvement in water supplies in rural and urban areas has considerably assisted the control of water-borne diseases. The Institute of Health in Singapore was completed in 1958 with assistance of a grant of 1,500,000 dollars from Colonial Development and Welfare Funds, and during 1958, 144 medical students and 5 dental students from the Colonial territories were placed by the Colonial Office in teaching schools in the United Kingdom and the Irish Republic. Several overseas governments are now contributing to the Applied Nutrition Unit at the London School of Hygiene and Tropical Medicine.

* Colonial Office. The Colonial Territories, 1958–1959. Pp. xxv+199. (Cmnd. 780.) (London: H.M. Stationery Office, 1959.) 10*s.* 6*d.* net.

GERM-FREE VERTEBRATES: PRESENT STATUS

IN a recent publication by the New York Academy of Sciences résumés are given of a series of papers delivered at a conference held by the Academy during May 1958*. It is divided into five parts: the first and second are concerned with instrumentation and with rearing of germ-free vertebrates; the third is concerned with the biological characteristics of germ-free animals; the fourth and fifth describe ways in which germ-free animals have been successfully used as a tool to solve otherwise intractable problems and discusses future possibilities in this direction.

It has taken thirty years of technical development to produce germ-free animals of most laboratory species and thereby refute the opinion of Pasteur that germ-free animals would not survive. Although the methods adopted are subject to continuous improvement, simplification and standardization, they remain expensive and complex. Nevertheless, the time has come when germ-free animals are produced on a limited scale for practical research projects and, since the maintenance of the germ-free state over a limited time is much simpler than the production of germ-free animals, a plea is made for wider support for centres to produce and distribute them. Study of the physiology and nutritional requirements of germ-free animals is a necessary preliminary to their use in research. In this connexion, it is noted that their lymphoid tissue is immature compared with that of conventional animals. Plasma cells are present in small numbers and the amount of serum γ-globulin is much reduced.

* Annals of the New York Academy of Sciences. Vol. 78, Article 1: Germfree Vertebrates: Present Status. By James A. Reyniers and 30 other authors. Pp. 1–400. (New York: New York Academy of Sciences, 1959.) 5 dollars.

Germ-free animals are free from antibacterial agglutinins readily demonstrable in conventional animals, although in some instances antibodies are later developed due to antigen in the autoclaved diet. Undoubtedly germ-free animals will provide a useful tool for immunologists in such questions as the source of 'natural antibodies' or whether serum γ-globulin exists apart from that produced in response to an antigenic stimulus. Indeed, germ-free animals raised on diets free from antigenic materials would be a boon to immunologists. In one paper, germ-free animals were indispensable in experiments to refute the hypothesis that irreversible hæmorrhagic shock was due to absorption of bacterial endotoxins from the bowel.

Interesting experiments are described to show that the increased growth-rate of conventional chicks, reared on diets containing penicillin, did not occur in germ-free chicks, but did occur after particular bacterial species had been experimentally established in their intestines. Another paper gives an account of experiments which show that infection with *Entamoeba histolytica* produces no lesions in germ-free guinea pigs, whereas it produces extensive ulceration of the intestinal mucosa in conventional guinea pigs. The potential value of germ-free animals in investigation of dental caries is, of course, obvious. In addition, the process of ageing in germ-free animals offers possibilities, but very little has been achieved so far in this respect on account of competition for available germ-free animal accommodation. However, perhaps the most exciting possibilities of germ-free animals at present relate to investigation of latent viruses, cancer and the relationship between them. No work upon latent viruses with germ-free animals is reported but a beginning has been made with cancer.

Filtrates from methylcholanthracene-induced tumours in conventional chicks are not infective, but when such tumours are induced in germ-free chicks inoculation of cell-free suspensions, produced by filtration through coarse filters, produces tumours and inoculation of suspensions produced by filtration through fine filters produces a fatal wasting disease. Purification procedures and electron microscopy tend to associate the tumour-producing activity with microsomal or virus-like particles of 50–150 mμ diameter and the wasting disease with smaller particles (20 mμ). In uncompleted experiments with germ-free C_3H mice, no mammary tumours in the females or hepatomas in the males have appeared in 100 mice of which the oldest are more than 700 days, whereas in comparable conventional C_3H mice 90 per cent die of these tumours within about 300 days.

It seems that germ-free experimental animals are likely to prove indispensable tools to answer certain basic questions of capital importance in several fields of biological research; yet after these questions have been answered the value of germ-free animals may perhaps decline. This poses a difficult problem for those called upon to decide whether to support the production of germ-free animals, an expensive and long-term undertaking. A. W. Gledhill

WATER POLLUTION RESEARCH, 1958*

Applied scientific research must ensure that projects and results are directly relevant and immediately available, so it is satisfactory to see from the annual report of the work of the Water Pollution Research Laboratory that contacts with the industries and authorities concerned are increasing and producing useful conclusions. Apart from the report itself, information is exchanged through numerous publications in journals, the invaluable *Water Pollution Abstracts*, a new series entitled "*Notes on Water Pollution*", and several committees recently established where the Laboratory makes close contact with the River Boards, the Institute of Sewage Purification and the Federation of British Industries. Financial support by the latter body has enabled two new investigations of considerable importance to industry to be started, and there are many examples in the report of valuable short-term studies undertaken in collaboration with specific industries, often on a repayment basis. In particular, suggestions made by the Laboratory have enabled several firms to reduce the danger of pollution by simple changes in manufacturing processes instead of expensive purification plant, and anaerobic digestion has been shown to be suitable for the treatment of slaughterhouse wastes but not for whisky wastes.

In the field of automatic apparatus it is anticipated that the merits of the dissolved oxygen recorder developed and applied by the Laboratory will be recognized by commercial production. The use of continuous recorders for oxygen and temperature has made it clear that isolated samples often do not give sufficient information for the accurate assessment of the condition of a river.

The studies made in the Laboratory of the foaming of detergents, and their behaviour in sewage treatment plant, are of particular interest. As the detergents normally used are resistant to biological treatment in percolating filters or activated sludge plant, the effluents may cause objectionable frothing in rivers. Recently, a new active material has been developed by the industry and tests have shown that a much greater proportion is destroyed during sewage treatment. If this is confirmed in large-scale trials, and if it could then replace existing detergents, the state of many rivers and drinking-water supplies should be improved directly. There might also be a general improvement in the quality of many effluents, as the efficiency of treatment can be affected by the amount of detergent present.

It is to be hoped that successes in empirical investigations will not distract attention from more prolonged and fundamental studies which are likely to be more economical in the long run. For example, the detailed results of the analysis of domestic sewage are proving to be very interesting and it will be extremely useful to know exactly the nature of the substances which treatment must aim to destroy. Biologists will be encouraged to see the success of modern analytical methods for the detailed separation and identification of the components of such a complex material.

* Department of Scientific and Industrial Research. Water Pollution Research 1958: The Report of the Water Pollution Research Board with the Report of the Director of the Water Pollution Research Laboratory. Pp. v+113+4 plates. (London: H.M. Stationery Office, 1959.) 7s. net.

Investigations of the toxicity of various substances and the effects of temperature on fish continue. In these studies, and also in work on biological treatments of sewage, cyanides and phenols, it is interesting to see the growing significance of acclimatization of the organism or the community. Most of the toxicity studies have been based on mortalities during relatively short periods of exposure, and it would now seem necessary to study the effects of much longer exposures on the growth, longevity and reproduction of fish.

The survey of the Thames estuary, which has been an important part of earlier reports, is now nearing completion. Some examples of the difficulties that arise in predicting the behaviour of this intricate system are to be found in this report. Recent improvements in the treatment of some of the sewage discharged to the estuary have apparently not produced the expected improvement in estuarine conditions for much of the year. It seems probable that dredging may have to be added to the numerous factors which must be considered, as it appears that a concurrent reduction in the removal of organic matter in this way could have masked the effects of the improvements.

It may be helpful to direct attention to two errors which might lead the reader to erroneous conclusions. In Fig. 4, p. 13, the continuous lines refer to a depth of 3 ft. and the broken lines to 15 ft., not vice versa as in the key given. In a section describing the work of the reviewer while at the Laboratory (p. 85), the last paragraph should start, "The gross rates of oxygen production are nearly twice (not ten times) published values for various aquatic plants in optimum light but in stagnant vessels. Static values for the plants used, obtained by extrapolating the flow curves, are much lower than the experimental stagnant results". D. F. WESTLAKE

UNIVERSITY OF OXFORD SECOND TANGANYIKA EXPEDITION

WITH the encouragement of the Government of Tanganyika, a second party from the University of Oxford visited the Mahali Mountains on the eastern shore of Lake Tanganyika during July to October to continue the work started by last year's expedition (see *Nature*, **183**, 726; March 14, 1959).

The Mahalis form a small, well-defined, yet little known region, not typical of most of Tanganyika, where flora and fauna of East and West Africa meet. Whereas last year's expedition concentrated on the southern half of the peninsula, this year the base camp was farther north, at Kasoge, a short distance from Mount Kungwe (c. 8,250 ft.), the highest peak in the range.

A collection of plants at all altitudes above lake-level was made by R. M. Harley and J. G. B. Newbould, of the Botany School, Oxford. The specimens have been sent to the Royal Botanic Gardens at Kew for identification and will be used in the preparation of the Flora of Tropical East Africa. The collection includes a number of live orchids which are now in the orchid houses at Kew.

The two zoologists made a general collection of birds, snakes and small mammals and also specialized in Arachnida and freshwater biology. J. A. Cooke, of the Zoology Department, Oxford, made an exhaustive study of the spiders in the area and collected nearly 4,000 specimens. D. H. Eccles, of the Bureau of Animal Population, Oxford, collected several hundred fish from the streams draining into Lake Tanganyika, using both poison and electric shock methods. For two weeks the party was joined by G. H. Yeoman, of the Tanganyika Veterinary Department, who was investigating parasites on small mammals.

W. J. Wadsworth and R. C. Herrera, of the Department of Geology, Oxford, made a geological investigation of the layered igneous intrusion at Kapalagulu, about 20 miles north-east of Mount Kungwe. T. E. Stevens, of the same Department, completed a survey of the Mount Kungwe region itself. Collections of rock specimens from these two areas will be examined in the Department of Geology, Oxford, and at the Geological Survey, Dodoma, Tanganyika.

R. Davis, of the Institute of Experimental Psychology, Oxford, conducted experiments on reaction times and visual perception among local inhabitants and, tested 350 children in missions and native authority schools as part of a comparative study of African and European children. A household survey of villages in the northern half of the peninsula was also undertaken.

The members of the expedition participated in the social activities of the region and various features of local life were recorded, both photographically and on magnetic tape. R. DAVIS

GEOMAGNETIC ACTIVITY AT HALLEY BAY ON DISTURBED DAYS

By J. MACDOWALL
Meteorological Office, London

THIS examination of the diurnal variation of geomagnetic activity at Halley Bay, lat. 75° 31′ S., long. 26° 37′ W., was based on Q-indices which occurred during the five most disturbed days in each month. A year was formed from the twenty months observations and then divided into four seasons.

Greatest activity occurred for the seven hours after local midnight in each season, but a significant seasonal change occurred in the form of the daily variation.

Fig. 1 shows that the mean activity of the year was of a similar form to the diurnal variation at the equinox. However, Fig. 2 illustrates the marked seasonal change in the form of the diurnal curve. Near local midnight, activity started earliest in winter and latest in summer, but activity after mid-

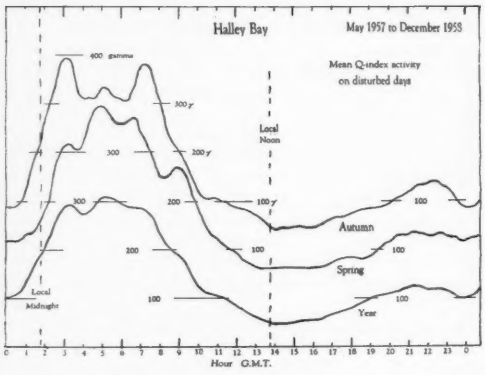


Fig. 1. The general form of the variation was similar in these periods

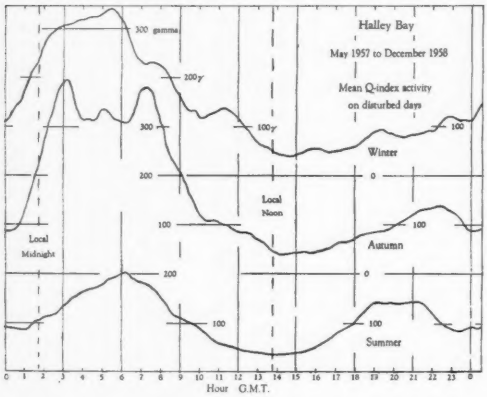


Fig. 2. Seasonal changes to the daily variation of geomagnetic activity

night was greatest in autumn and least in summer. At 1115 G.M.T. there was a subsidiary maximum of activity in the winter: this effect was just evident at 1015 G.M.T. in autumn, but during summer there was no sign of this feature.

For most of the year the second most active period occurred in the evening and was separated from the main disturbance period by a brief minimum near 0000 G.M.T. Such evening activity was greatest and earliest in summer, and the equinox showed an intermediate type of variation. In the later aspect the seasonal change in the evening differs from the change in the early morning, where greatest activity occurred at the equinox. Further difference between the early morning and evening periods was revealed when the change with time in the sign of 'bay-like' disturbances (of types *b*, *bp*, *bps*, *bs*) was examined in Table 1.

Table 1. EFFECT OF TIME ON THE MAIN MOVEMENT OF THE HORIZONTAL AND VERTICAL COMPONENTS IN BAY DISTURBANCES, HALLEY BAY, JANUARY–JUNE 1958

Percentage of bay disturbances with certain signs

G.M.T. period	H −ve Z +ve	H +ve Z −ve	Other signs	Total number all movements
15–18	0	100	0	5
18–21	3	82	15	31
21–24	43	24	33	51
0–3	93	1	6	78
3–6	93	0	7	56
6–9	93	0	7	14
9–12	100	0	0	5
Day	70	18	12	240

During the most active period from 0000 to 0600 G.M.T., field changes of such geomagnetic phenomena were almost entirely negative for the horizontal component and positive for the vertical component, but in the subsidiary active period before 0000 G.M.T. the reverse field movements were most frequent. The zone of maximum auroral frequency was situated just south of Halley Bay, so that the sense of the change of vertical force would indicate a weakening of the auroral zone electro-jet before 0000 G.M.T., which was followed by a recovery and an even greater strengthening in the early morning. The change of the horizontal component indicated a reversal in the direction of the overhead zonal disturbance current system at about 0000 G.M.T.

Other ionospheric effects were observed with a Union ionosonde; in particular, ionosonde blackout observations bore a different relation to *Q*-indices in the evening and early morning. In the winter of 1958, blackouts which occurred from 1900 to 2300 G.M.T. were not associated with high *Q*-indices, whereas during blackouts from 0000 to 1500 G.M.T. high *Q*-indices occurred. This relation is illustrated in Fig. 3 with the aid of ionosonde blackout data kindly supplied by Mr. W. H. Bellchambers. It is

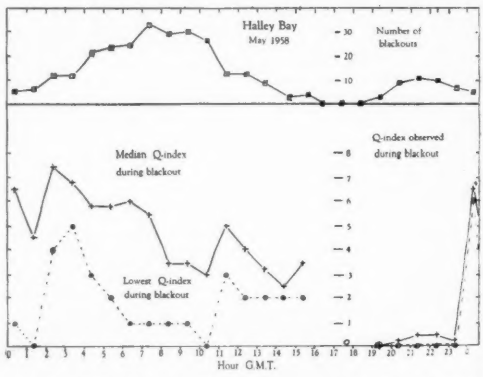


Fig. 3. Geomagnetic activity which occurred during Union ionosonde blackout conditions

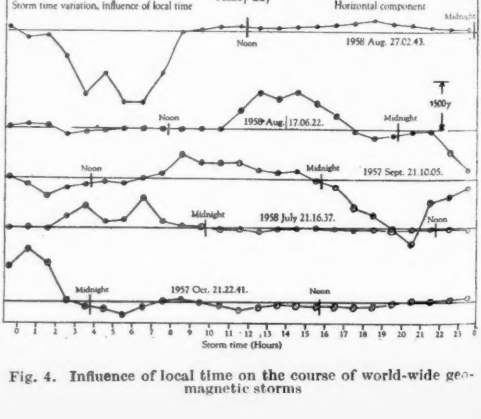


Fig. 4. Influence of local time on the course of world-wide geomagnetic storms

also of interest to note in Fig. 3 the special nature of this relation near 1100 G.M.T., which may be compared with the peak in Q activity at 1115 G.M.T. in winter and shown in Fig. 2.

Nine occasions of confirmed world-wide sudden storm commencements (SSC) with commencements at different local times were examined. In Fig. 4 five of these storms are presented and show that the local time variations dominate the course of the storm. As in the case of bay disturbances, positive departures of the horizontal field occur before midnight and negative departures after midnight.

This work formed a part of the programme of the British National Committee for the International Geophysical Year; it was started at the Royal Society Base while I was seconded from the Meteorological Office. A full discussion of the results will be published as soon as possible. I gratefully acknowledge Mr. A. Blackie's assistance with the geomagnetic observations.

AN X-RAY STUDY OF RIGID-BODY AND INTERNAL VIBRATIONS OF ORGANIC MOLECULES

By Dame KATHLEEN LONSDALE, D.B.E., F.R.S., and Dr. H. JUDITH MILLEDGE

Department of Chemistry, University College, London, W.C.1

THE intensity of diffraction of X-rays by a set of parallel crystal planes depends upon the arrangement of the atoms, their electron-density distribution and their state of motion. Hitherto, most crystal-structure analyses have concentrated on the obtaining of accurate atomic positions and thence the spatial configuration of the molecules. It is almost always assumed that the atomic scattering factor f is a spherical function and that the atomic vibrations are at least approximately isotropic. In 1941 E. W. Hughes showed that it was impossible to obtain good agreement between observed and calculated scattering amplitudes for the various diffracted beams from crystals of melamine unless it was postulated that the vibration normal to the molecular plane was larger in amplitude than that parallel to the molecular plane. Moreover, electron-density Fourier diagrams often show elliptical atomic contours, which may indicate a static non-spherical distribution but are much more likely to indicate non-spherical motion. Fourier diagrams obtained from neutron-diffraction observations likewise show elliptical contours, and these must certainly indicate non-spherical movements (or at least displacements) of the nuclei. If the ellipticity persists at low temperatures then there is the possibility, of course, that it may indicate some kind of disorder or uncertainty in the atomic positions. The diffuse spots and streaks which form the elaborate patterns found on well-exposed X-ray photographs taken of stationary crystals using characteristic radiation are also due either to atomic or molecular thermal vibrations, or (if they are not variable with temperature) to zero-point energy or disorder. Finally, the large degree of anisotropy of thermal expansion usually found in organic crystals and easily measured accurately by X-ray diffraction methods proves that the lattice or molecular vibrations must also be anisotropic.

With the advent of high-speed computers, it is now possible to obtain for each atom, at least in the case of relatively simple molecules, not only the atomic co-ordinates but also sufficient constants to indicate the directions and magnitudes of the principal atomic thermal vibrations. (The details of the *Pegasus* computer programmes used in this work will be published elsewhere.) Making the simplifying assumption that the atomic vibration figure is an ellipsoid, this involves $(9n + 1)$ unknowns for a molecule containing n independent atoms: that is, we require for each atom three co-ordinates x, y, z; three principal atomic Debye factors B_X, B_Y, B_Z; three orthogonal directions to give the orientation of the atomic vibration ellipsoid, $\vec{X}$, $\vec{Y}$, $\vec{Z}$; and a scaling factor to put all diffraction amplitudes on an absolute scale. The accuracy with which these unknowns may be experimentally determined depends upon the number of available hkl diffraction intensities (these usually amount to several hundred, depending upon the size of the unit cell) and upon the precision achieved in making the intensity measurements. This can be tested by repetition, especially using more than one crystal, but is also dependent upon the success with which data can be scaled together when recorded on more than one film or under different conditions in respect of the geometry of the diffracted beams and the recording device. It is, in short, essential that systematic errors be avoided if an accurate determination of molecular and atomic vibrations is required.

Cruickshank[1] has pioneered in showing how to extract information about the translational and librational amplitudes of the molecule, treated as a rigid body, from the atomic thermal-vibration ellipsoids. He has, however, accepted Higgs's suggestion[2] that, compared with these rigid-body movements, the intramolecular vibrations, which distort the molecule, will be small.

We are able to prove that this is not always the case, and that if and when the internal vibrations are comparatively small, it may still be possible to deduce them from the experimental observations. Perhaps the most obvious proof is that even diamond, which is an infinite three-dimensional molecule, does show temperature-dependent diffuse spots (Lonsdale[3]), that it does expand (Thewlis and Davey[4]) and eventually becomes transformed into graphite. The root-mean-square $(\overline{u_X^2})^{1/2}$ amplitude of atomic vibration in diamond is about 0·04 A. (as deduced from the Debye temperature: Lonsdale[5] gives a value of $\overline{u^2} = 3\overline{u_X^2}$ which is too low) even though each atom is bonded to four others. Since $B_x = 8\pi^2 \overline{u_X^2}$, the value $(\overline{u_X^2})^{1/2} = 0{\cdot}04$ A. will correspond to a Debye factor of 0·13 (A.^{2}) and this, although small, is not negligible.

One of us (H. J. M.) has calculated, from 3-dimensional powder data kindly supplied by Dr. G. E. Bacon, the Debye factor for graphite [0001] and has

found the value 0·63, which corresponds to a root-mean-square vibration amplitude of about 0·09 A. Graphite has 'infinite' two-dimensional molecules which cannot undergo 'rigid-body' translations or librations. All vibrations must be intramolecular or distorting vibrations, and hence 0·09 A. gives some measure of the internal vibration amplitude that is possible for an atom tightly bonded to two others, the vibration being normal to the plane of the bonds. In this case, however, the anisotropy of the electron-density distribution and hence of the atomic scattering factor must certainly be taken into account, and this will be considered elsewhere. What is relevant here is that intramolecular thermal vibrations alone, without translations or librations, are responsible for the thermal-expansion coefficient of graphite, which between 0° and 800° C. is $28{\cdot}2 \times 10^{-6}$ (° C.)$^{-1}$ along [0001] (Nelson and Riley[6]).

A refinement of the hexagonal boron nitride (BN) structure, for which even with very careful intensity measurements Pease[7] only attained an R-factor of about 17 per cent, shows that by re-scaling and imposing anisotropic B-values of 0·7 in the molecular planes and 1·4 along the van der Waals [0001] bonds, R is reduced to 4·7 per cent. Certainly there is here also some bond anisotropy to be taken into account, but part, at least, of B [0001] must be due to thermal vibration and this value corresponds to a root-mean-square amplitude of intramolecular out-of-plane vibrations of 0·13 A. This larger magnitude as compared with the graphite out-of-plane vibrations fits well with the larger boron nitride thermal-expansion coefficient α [0001] = $40{\cdot}5 \times 10^{-6}$ (0–800° C.).

These coefficients of expansion, however, are small compared with those of most aromatic compounds (Lonsdale[8]), and we may undoubtedly expect that in the case of plane finite molecules separated from their neighbours by van der Waals distances, the principal cause of expansion will be the librations involving out-of-plane movements.

Our studies of a number of simple organic molecules have shown that, given a structure for which the residual-error factor R is small (preferably well below 10 per cent), it is possible to deduce, within limits, numerical values not only of the translational and librational vibration amplitudes but also of intramolecular amplitudes; and that the internal molecular vibrations that emerge from these analyses are reasonable ones. A similar analysis carried out on earlier Debye factors corresponding to higher R values (say of upwards of 12 per cent) simply produces nonsense. A useful check on the vibration pattern may be obtained by carrying out an independent structure and vibration analysis on the same compound at another temperature, or on an isomorphous or isostructural compound.

Following Higgs[2], we assume that the various molecular vibrations are independent of phase and that the resultant mean-square amplitude in any given direction is the sum of the mean-square amplitudes of atomic vibrations of all kinds, rigid-body or distortional, in that direction. For each molecule we may specify three principal directions of vibration, say $\vec{L}$, $\vec{M}$, $\vec{N}$ (Fig. 1), and for each atom in the molecule we have three principal directions $\vec{X}$, $\vec{Y}$, $\vec{Z}$ given by our X-ray analysis. These may be deduced from Fourier analysis (for example, $\vec{R}$, $\vec{T}$, $\vec{N}$ in 1.3.5-$C_6H_3Cl_3$) or postulated on grounds of symmetry and probability; thus, in di-*para*-xylylene, $C_{16}H_{16}$ (Brown[9]), the most likely $\vec{X}$, $\vec{Y}$, $\vec{Z}$ directions coincide at least approximately with the molecular $\vec{L}$, $\vec{M}$, $\vec{N}$. The actual determinations of $\vec{X}$, $\vec{Y}$, $\vec{Z}$ purely by computer refinement is the weakest link in the whole chain, being subject to very large standard deviations indeed. In fact, if one refines a molecule known to be *symmetrical* as if it were not (that is, using the computer programme for a less symmetrical space group) then the values produced for both B_X . . . and $\vec{X}$ . . . are found to be *asymmetrical*, especially in early stages of the refinement. But whereas the B_X . . . values for atoms related by symmetry converge (if the data are good) to a point where they may justifiably be averaged, the $\vec{X}$ . . . values do not necessarily do so. The machine, in fact, may attempt to improve the agreement between observed and calculated structure amplitudes by producing nonsense; and in the case of molecules having symmetrical formulæ an informed guess at the directions of $\vec{X}$, $\vec{Y}$, $\vec{Z}$ is probably better than an averaging of widely differing computer-produced values; although it may be advisable to try more than one such trial set of directions.

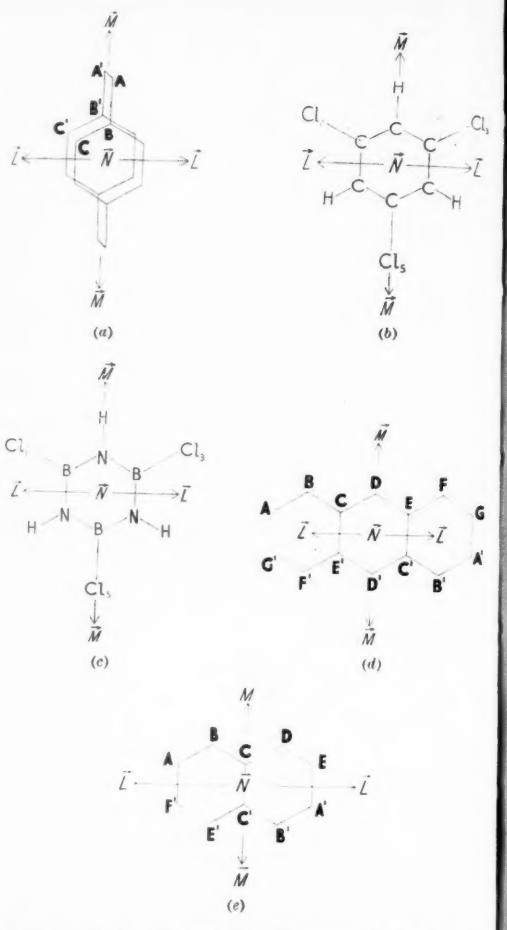


Fig. 1. Principal molecular directions assumed for $C_{16}H_{16}$, *symm.* $C_6H_3Cl_3$ ($C_6H_3Br_3$), $B_3N_3H_3Cl_3$, $C_{14}H_{10}$ and $C_{16}H_8$

We begin, therefore, with values of the molecular geometry and with three principal Debye factors B_X . . . for each atom, derived from a large quantity of diffraction data. Let us call the mean-square rigid-body translation amplitudes t_L^2, t_M^2, t_N^2 (A.^{2}) and the rigid-body libration amplitudes φ_L^2, φ_M^2, φ_N^2 (radians^2) and let us then obtain a set of equations for $\overline{u_X^2}$ ($= B_X/8\pi^2$) . . . for each atom **A**, **B**, **C** . . . in the molecule. The movement due to libration, although along an arc, may be, for small displacements, taken as being along a tangent normal to the perpendicular (length R) from the atom to the axis of libration. Its mean-square amplitude will be $R_L^2\,\varphi_L^2$ . . . (A.^{2}). We wish to find t_L, t_M, t_N ; φ_L, φ_M, φ_N ; and also $\mathbf{A}_X$, $\mathbf{A}_Y$, $\mathbf{A}_Z$, $\mathbf{B}_X$ . . . $\mathbf{C}_Z$ . . . which are the independent atomic vibration amplitudes along $\vec{X}$, $\vec{Y}$, $\vec{Z}$. The latter may be expected to combine into a few simple proper vibrations of the molecule. The whole system of vibration must explain the anisotropy of thermal expansion.

The details of the calculations will be published elsewhere, but it is possible to give here a selected example to illustrate the procedure. Consider $C_{16}H_{16}$, the molecule of which consists of two facing benzene rings linked by $(CH_2)_2$ bridges in *para*-positions[9]. The space group is $P4_2/mnm$ and the molecule (Fig. 1) has *mmm* symmetry. There are therefore only three independent atoms **A** (—CH_2—), **B** (—C<) and **C** (—CH=). The equations describing the $\vec{M}$ and $\vec{N}$ movements of these atoms are (at 291° K.):

$$\overline{u^2}(A,M) = t_M^2 + 0{\cdot}66\,\varphi_L^2 + \mathbf{A}_M^2 = 0{\cdot}0916 = 7{\cdot}24/8\pi^2 \quad (1)$$

$$\overline{u^2}(B,M) = t_M^2 + 1{\cdot}89\,\varphi_L^2 + \mathbf{B}_M^2 = 0{\cdot}0737 = 5{\cdot}82/8\pi^2 \quad (2)$$

$$\overline{u^2}(C,M) = t_M^2 + 2{\cdot}38\,\varphi_L^2 + 1{\cdot}42\,\varphi_N^2 + \mathbf{C}_M^2 = 0{\cdot}0946 = 7{\cdot}47/8\pi^2 \quad (3)$$

$$\overline{u^2}(A,N) = t_N^2 + 7{\cdot}98\,\varphi_L^2 + \mathbf{A}_N^2 = 0{\cdot}0753 = 5{\cdot}95/8\pi^2 \quad (4)$$

$$\overline{u^2}(B,N) = t_N^2 + 1{\cdot}91\,\varphi_L^2 + \mathbf{B}_N^2 = 0{\cdot}0547 = 4{\cdot}32/8\pi^2 \quad (5)$$

$$\overline{u^2}(C,N) = t_N^2 + 0{\cdot}51\,\varphi_L^2 + 1{\cdot}42\,\varphi_M^2 + \mathbf{C}_N^2 = 0{\cdot}0619 = 4{\cdot}89/8\pi^2 \quad (6)$$

From (1) and (2) we have :

$$\mathbf{A}_M^2 = 0{\cdot}0179 + \mathbf{B}_M^2 + 1{\cdot}23\,\varphi_L^2$$

and therefore $\mathbf{A}_M^2 > 0{\cdot}0179$ (A.^{2}) ; $\mathbf{A}_M > 0{\cdot}13$ A.

(The estimated standard deviation in $\overline{u_X^2}$ is about 0·006 A.^{2}, corresponding to 0·5 in $\mathbf{B}_X$.) From (4) we know that $7{\cdot}98\,\varphi_L^2 \leqslant 0{\cdot}0753$ (t_N^2, $\mathbf{A}_N^2$ are zero or positive) and from (5) $t_N^2 \leqslant 0{\cdot}0547$ (φ_L^2, $\mathbf{B}_N^2$ zero or positive). Both these equalities could not, of course, be true simultaneously. From (4) and (5):

$$6{\cdot}07\,\varphi_L^2 = 0{\cdot}0206 + (\mathbf{B}_N^2 - \mathbf{A}_N^2)$$

A large value of $\mathbf{A}_M$ would involve considerable stretching of bonds, unless $\mathbf{B}_N$ and $\mathbf{C}_N$ also have medium to large positive values ; and therefore it is probable that $\mathbf{B}_N^2 > \mathbf{A}_N^2$, since $\mathbf{A}_N$ would not be large for any reasonable molecular vibration. If $\mathbf{B}_N^2 > \mathbf{A}_N^2$, then :

$$0{\cdot}0094 > \varphi_L^2 > 0{\cdot}0034$$

which gives $\mathbf{A}_M^2 - \mathbf{B}_M^2 > 0{\cdot}0221$; $\mathbf{A}_M > 0{\cdot}15$ A. and

$$t_M^2 < 0{\cdot}0673 \qquad t_M < 0{\cdot}26 \text{ A.}$$
$$t_N^2 < 0{\cdot}0482 \qquad t_N < 0{\cdot}22 \text{ A.}$$

By a series of similar approximations we arrive at the values shown in Table 1, the limits of error being based on the indeterminacy inherent in the argument and not on possible experimental errors in the $\overline{u_X^2}$. This means that the actual numerical values may still need adjustment, although we believe the general pattern to be a correct one.

Table 1. Probable Ranges of Values of Intermolecular and Intramolecular Root-Mean-Square Vibration Amplitudes for Di-*para*-xylylene Molecules at 291° K. and 93° K., respectively

	t_L	t_M	t_N	φ_L	φ_M	φ_N
291° K.	> 0·16	0·235	0·14 A.	0·065	0·045	0·065 radians
	< 0·21	0·255	0·21	0·085	0·08	0·085
93° K.	> 0·14	0·15	0·09 A.	0·045	0·03	0·03 radians
	< 0·16	0·16	0·14	0·065	0·065	0·05

	$\mathbf{A}_L$	$\mathbf{B}_L$	$\mathbf{C}_L$	$\mathbf{A}_M$	$\mathbf{B}_M$	$\mathbf{C}_M$
291° K.	> 0·10	0·07	0	0·14	0	0·07 A.
	< 0·17	0·12	0·10	0·17	0·06	0·12
93° K.	> 0·08	0·045	0	0·095	0	0·065 A.
	< 0·13	0·07	0·065	0·11	0·045	0·08

	$\mathbf{A}_N$	$\mathbf{B}_N$	$\mathbf{C}_N$
291° K.	> 0	0·07	0·10 A.
	< 0·06	0·14	0·20
93° K.	> 0	0·045	0·045 A.
	< 0·045	0·10	0·125

The rigid-body librations are such that the maximum values of $R_X\varphi_X$ are about equal in magnitude to the translational amplitudes. They are reduced by about one-third at 93° K. as compared with room temperatures, but the general pattern is the same although the complete structure analyses at the two temperatures are entirely independent (Lonsdale, Milledge and Rao, *Proc. Roy. Soc.*, in the press).

The internal movements are such as to imply two principal distorting vibrations of the molecule (Fig. 2). The first is a concertina movement involving $\mathbf{A}_M$ and $\mathbf{C}_N$, with smaller movements of $\mathbf{B}_M$, $\mathbf{B}_N$. The second is a twisting movement involving $\mathbf{A}_L$ and $\mathbf{B}_L$ with smaller $\mathbf{C}_L$ and $\mathbf{C}_M$ vibrations. There will be other movements superimposed on these, but only

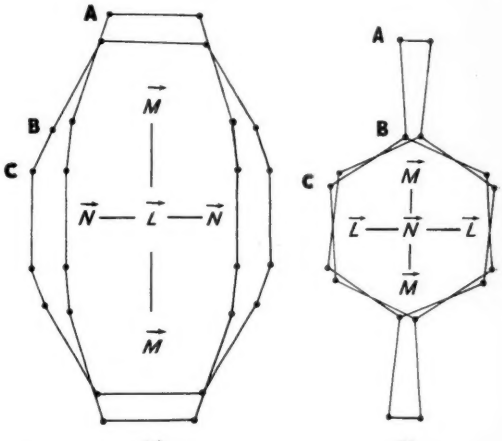


Fig. 2. Molecular vibrations found for di*para*xylylene, $C_{16}H_{16}$

Table 2

	$C_6H_3Cl_3$ (293° K.)	$C_6H_3Cl_3$ (93° K.)	$C_6H_3Br_3$ (room temp.)	$B_3N_3H_3Cl_3$ (room temp.)
t_L (A.)	0·195	0·115	0·155	0·24
t_M (A.)	0·16	0·07	0·13	0·20
φ_N (radians)	0·085	0·03	0·05	0·065

these are clear ; and it will be seen that they involve amplitudes comparable with, though smaller than, the t and $R\varphi$ values. We are investigating other compounds (*bis*-2.7-dimethylene naphthalene and di-*m*-xylylene) where a forced proximity and parallelism of aromatic rings may be expected to imply large internal vibrations of the molecule.

We have also studied 1.3.5-$C_6H_3Cl_3$ (at 293° K. and 93° K.) and 1.3.5-$C_6H_3Br_3$ (Milledge and Pant[10] ; Milledge and Lonsdale, in the press) and we have refined the structure of *BBB*-$B_3N_3H_3Cl_3$ originally analysed by Coursen and Hoard[11]. The first two are isomorphous, the latter has a structure closely similar in orientation of the molecules, although not isomorphous (Lonsdale, in the press). The data for the benzene compounds are insufficient to give a complete vibration analysis, but they do give the approximate values of t_L, t_M and φ_N shown in Table 2.

It will be seen that the magnitudes are comparable with those obtained for $C_{16}H_{16}$, but that the heavier isomorph has the smaller vibrations.

The independent vibrations of the three chlorine atoms in $C_6H_3Cl_3$ are not equivalent. In a direction tangential to the benzene ring the atoms Cl_1 Cl_3, Cl_5 (Fig. 1) have mean-square amplitudes 0·0136 ; 0 ; 0·0324 A.^{2}, respectively, at 293° K. (or possibly $0{\cdot}0136 + k$; k ; $0{\cdot}0324 + k$, with a corresponding reduction in φ_N[2]. A weakness of our simple method is that it cannot distinguish these two possibilities). To eliminate these differences would involve errors of 1·0 and 2·5, respectively, in the measurement of the Debye factors, whereas the estimated standard deviation is only about 0·25. Nor is it unreasonable that they should differ, since the bonds linking them to neighbouring molecules are different for the three chlorine atoms.

The molecule of $B_3N_3H_3Cl_3$ has a plane of symmetry parallel to (010). The chlorine atom on the mirror plane has $\overline{u_T{}^2}$ (tangential to the ring) greater than that of the asymmetric chlorine. But there are also indications of a marked independent movement of the boron and nitrogen atoms normal to the molecular plane. This is consistent with the [0001] movements in hexagonal boron nitride previously mentioned.

Finally, we may consider anthracene and naphthalene. Previous thermal analyses of anthracene have been based on experimental data which were only claimed to be accurate to about 10 per cent (Mathieson *et al.*[12]) and which more accurate recent data have shown to be subject also to some systematic errors. No complete set of accurate 3-dimensional $|F|$ values is yet available, but two partial sets, one 3- and one 2-dimensional, measured by Geiger-counter and photographic methods respectively, agree to 3·5 per cent and refine to 4·4 and 5·6 per cent, to give molecular geometries and B_X values which differ by far less than the estimated standard deviations calculated for either set separately. Moreover, independent two-dimensional data at low temperatures refine to 6·6 per cent to give a similar, though reduced, thermal vibration pattern.

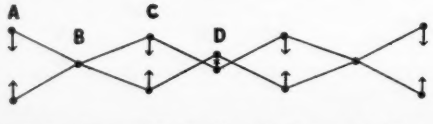


Fig. 3. One possible vibration of anthracene, $C_{14}H_{10}$

Cruickshank[1] gave the thermal amplitudes for each atom A—G (Fig. 1) in tangential, radial and normal directions with respect to the mass centre and plane of the molecule. We believe that the experimental data indicate that $\vec{L}$, $\vec{M}$ and $\vec{N}$ are the correct principal atomic directions, except possibly for the extreme atoms **A** and **G**. Assuming that **A** and **G**, **B** and **F**, **C** and **E** are equal within each pair, we obtain the results given in Table 3 (Lonsdale, Mason and Milledge, in preparation).

Table 3. Probable Ranges of Values of Intermolecular and Intramolecular Root-Mean-Square Vibration Amplitudes for Anthracene Molecules at 290° K. and 95° K. respectively

	t_L	t_M	t_N	φ_L	φ_M	φ_N
290° K.	> 0·22	0·16	0·155 A.	0·06	0·045	0·05 radians
	< 0·225	0·19	0·17	0·07	0·05	0·06
95° K.	> 0·125	0·05	0·08 A.	0	0	0·02 radians
	< 0·135	0·085	0·11	0·03	0·03	0·035

	A_L	B_L	C_L	D_L	A_M	B_M	C_M	D_M
290° K.	> 0·075	0·04	0	0	0	0 085	0	0·11 A.
	< 0·085	0·045	0·03	0·03	0·07	0·115	0·09	0·155

	A_N	B_N	C_N	D_N	
290° K.	> 0·06	0	0·05	0	A.
	< 0·09	0·03	0·075	0·05	

	A_L	B_L	C_L	D_L	A_M	B_M	C_M	D_M
95° K.	> 0·04	0·02	0·04	0	0	0·095	0	0·085 A.
	< 0·055	0·04	0·055	0·02	0·085	0·105	0·06	0·11

	A_N	B_N	C_N	D_N	
95° K.	> 0·03	0	0·055	0	A.
	< 0·10	0·03	0·09	0·07	

The t and φ values are similar to those of the compounds previously studied, but there are also striking intramolecular vibrations. The large values of B_M and D_M, combined with A_L, point to an in-plane distortion which appears not to be markedly sensitive to temperature. Similarly, the out-of-plane vibrations A_N and C_N are large at both temperatures, but B_N and D_N are not. If A_M and C_M are related in phase, then some such movement as is shown in Fig. 3 would result ; but there is no proof that this particular interpretation of Table 3 is correct.

A weakness of the method previously mentioned, which may be illustrated by naphthalene, is that there is as yet no way of deciding, on our simple model, whether the calculated value of (say) t_L should be reduced by k and the values of A_L, B_L, etc., all increased by the same amount. Since A_L, B_L, etc., are not necessarily in phase, these would represent different vibration patterns. Thus, the observed $\overline{u_L{}^2}$ and $\overline{u_M{}^2}$ for naphthalene can all be satisfactorily explained in terms of $t_L = 0{\cdot}21$ A., $t_M = 0{\cdot}17$ A., $\varphi_N = 0{\cdot}072$ radians, with $A_L = A_M = B_L \ldots = 0$. In the perpendicular direction, however, A_N and C_N are found to be about $0{\cdot}11 \pm 0{\cdot}02$ A., with $B_N = 0$, implying an out-of-plane bending or puckering vibration of the molecule. Our consistent practice has been to assume that, failing evidence to the contrary, the internal vibrations are zero.

We recognize that the analyses we have illustrated here are over-simplified. The major defect, that of an assumption of a spherical atomic scattering factor,

is now being investigated in the light of the experimental data for graphite and hexagonal boron nitride. We do not believe that this source of error is eliminated by using only high-angle reflexions in the thermal analysis; errors may even be introduced by this procedure.

Perhaps we can justify the simplicity of our approach by suggesting that if earlier workers had been inhibited, in their initial analyses of the structures of simple ionic compounds and of diamond, by their original neglect of refraction, dispersion, temperature vibrations, defect and disorder, the fundamental advances in crystal-structure analysis for which they were responsible would have been very much delayed.

We are most grateful to several colleagues for the use of intensity data prior to publication, or for original data published in a modified form; and also for crystal specimens.

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One of us (K. L.) would like here to acknowledge personal indebtedness to the late Sir William Bragg for a training which emphasized that a simple approach to a difficult problem is not necessarily an inaccurate one.

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FLORAL MORPHOLOGY AND THE TAXONOMY OF EUCALYPTUS

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THERE has been, in recent years, a revival of interest in 'old-fashioned' comparative morphology as a tool in problems of taxonomy and phylogeny of Angiosperms[1]. Among the many groups in which conventional study of the morphology of the flower is still required is the large and important genus *Eucalyptus*, despite the fact that taxonomic relationships within this genus have been under fairly continuous scrutiny over the past 150 years. In this, as in certain other genera of Myrtaceae[2], the perianth parts are united to form one or two opercula which cover the flower until anthesis. Practically no reliable information is available concerning the structure and behaviour of the opercula in most species, and little use has been made of operculum characters in classification, apart from length and shape. Only one floral character has been used in delimiting most of the major categories within the genus, namely, the shape and mode of dehiscence of the anthers, and even here diversity has not been thoroughly exploited. No reference, for example, has ever been made to the presence of supernumerary glands (additional to the terminal gland) in the connective or on the anther lobes of certain species. Attempts have been made to produce a workable classification or deduce affinities between species using criteria such as bud and fruit shape, operculum length, shape, venation and glaucousness of leaves, cotyledon shape, and, more recently[3], seed anatomy. Such are the difficulties of the standard classification that, although this is put forward in what purports to be a 'key' to the genus[4], it is virtually impossible to use it as such; its deficiencies are well known to many Australian botanists (see, for example, ref. 5).

Comparative studies on the anatomy of wood[6], bark[7], chromosome complements[8], and pollen grains[9] have not yielded information of much value to the taxonomist, principally because these studies have been inadequate in both extent and depth. Nevertheless, botanists and foresters have become familiar with the facies of the species (more than five hundred according to Blakely, but possibly reducible to between three and four hundred) and this has led to the establishment of certain groups ('series') some of which may be accepted as consisting of closely allied species and therefore 'natural'. Others of Blakely's series are clearly artificial, and a few species are so imperfectly known that their allocation to series is either intuitive or quite arbitrary.

One of the worst features of *Eucalyptus* taxonomy is that it is based largely on what may be termed 'shaggy dog' features, characterized by the qualification 'more or less', rather than on quantitative features, expressible numerically or by presence or absence. But more important still is the lack of fundamental concepts of the relationships between the series. The larger taxonomic units or 'sections' are still based primarily on Bentham's five anther classes[10], despite the fact that at least forty kinds of anthers, differing in size, shape, dehiscence, etc.[11], have been distinguished, and that many species are notoriously difficult to 'key out' by their anthers. Any classification based entirely on shapes which can vary[11] and which cannot be adequately described in words is bound to contain anomalies and, as Bentham was well aware, such a classification, based on a single feature, must be somewhat artificial.

The genus *Eucalyptus* is supposed to be distinct from *Angophora* in that *Angophora* has both petals and sepals free, whereas in *Eucalyptus* one at least of the perianth whorls is in the form of an operculum which is shed at anthesis. In fact, as we shall show, certain species have only a petaline operculum with or without sepals, and the others have two opercula, the outer, sepaline, operculum being shed either prematurely or with the inner one at anthesis.

(We understand that Mr. L. A. S. Johnson, New South Wales Herbarium, has reached conclusions which, in certain details, confirm our own.) In certain species the sepals remain small but free from each other and may persist even on the mature fruit. This condition of chorisepaly is found in species belonging to several of the groups which have evolved within the genus, although certain chorisepalous species, mostly grouped as series Eudesmieae, may be fairly closely related to each other. The Eudesmieae are characterized by the persistence of the sepals as small teeth and/or the grouping of the stamens into four bundles, opposite the petals, which are fused to form a single operculum. In the very young flower bud the sepals are large, relative to the other organs, but they soon cease growth and in the mature flower seem comparatively minute. Some of the species of Eudesmieae have both persistent, free sepals and grouped stamens (for example, *E. tetragona*), others (for example, *E. gamophylla*, wrongly placed by Blakely) have only the first of these characters, and other species again have no sepals but have the stamens grouped (for example, *E. baileyana*). (For *Eucalyptus*, Blakely's nomenclature (see ref. 4) is used throughout this communication. For related genera, the nomenclature is that used in ref. 9.) Microscopic examination of sections of the flower buds of the latter species has failed to reveal any trace of the sepals. In all species of *Eucalyptus* the inner and outer surfaces of both sepals and petals are covered by a cuticle which can be distinguished by the techniques of fluorescence microscopy[12]. In critical cases, as, for example, in *E. albens*, the presence of two opercula, closely adherent to each other, can be demonstrated only by fluorescence microscopy of thin sections of the mature bud, or by a study of the development of the flower bud (Fig. 1).

The species of the Eudesmieae may be arranged as a series in which the sepal primordia are progressively reduced in size until they are eventually suppressed altogether, thus leading to a condition in which only one perianth whorl (presumably the corolla) is formed. This condition is common to most, but by no means all, of the species included in the section Renantherae, and to all of those in the Renantheroideae. The species of Blakely's series Myrtiformes (section Renantherae), *E. deglupta*, *E. raveretiana* and *E. brachyandra*, have double opercula and should possibly be placed with the Exsertae (section Macrantherae).

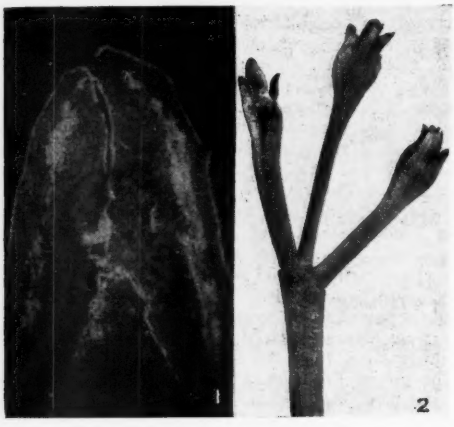

Fig. 1. Fluorescence photomicrograph (× 25) of a median longitudinal section of the tip of a mature flower bud of *E. sideroxylon*, showing fluorescence of cuticle on sepaline and petaline opercula and the cuticle-lined canal at the tip of the sepaline operculum. The canal in the petaline operculum is not seen in this section

Fig. 2. Young inflorescence (× 1·5) of *E. steedmanii* after shedding of bracts. The sepals of the three flower buds are free and comparatively large

We have already shown that *E. microcorys* should be excluded on other grounds[13], and both it and *E. guilfoylei* should be placed with the Transversae. On the other hand, *E. jacksoni*, which has been confused with *E. guilfoylei* (they are ecologically associated and were both originally described from non-flowering material) should be placed in the Renantheroideae near *E. patens*, as suggested by Gardner[14].

Some of the species of the Macrantherae are mono-operculate (*E. preissiana* and *E. megacarpa* (series Globulares) and *E. miniata* and *E. phoenicea* (series Miniatae). If these species are to be grouped together with the renantherous species, the new arrangement would appear to cut directly across the system based on anther shapes. The species involved in this grouping, however, show every transition from the macrantherous condition (Miniatae) to the fully renantherous. Moreover, other characters such as the possession of stellate hairs by the juvenile foliage and, according to recent work[3], some of the characters of the seeds appear to be common to many of the mono-operculate species and uncommon in, or even quite absent from, species with double opercula. We have traced[13] the progressive increase in the number of flowers in the dichasial cluster from the Eudesmieae to the Renantherae, and in the degree of fusion of the bracts of the involucre, which never number more than six in the mono-operculate species. We propose to group these species in a sub-genus for which we suggest the informal name 'Monocalyptus'.

Although asepaly or chorisepaly is necessarily associated with a mono-operculate condition, it does not follow that all species in which the sepals are free from each other should be placed together, and Blakely's Eudesmieae may prove to contain some species unrelated to the others. Chorisepaly occurs in some other species but has not hitherto been recognized. The young flower buds of *E. steedmanii*, a species described in 1933, has sepals which are very large in proportion to the other organs. Although they remain free from one another, they become adherent to the petaline operculum as thick ribs with free recurved tips (Fig. 2). This species appears to be most closely allied to those of the series Dumosae. Free sepals are also present on the flower buds of *E. kruseana*, but they are delicately attached and are usually shed a little before anthesis (Fig. 3). This species is placed by Blakely in a highly artificial series, *Globulares*, but in its habit, foliage and cotyledon shape[15] it resembles *E. crucis* and should be placed near it.

The primordia of both sepals and petals arise in decussate pairs (in accordance with the basic phyllotaxis of *Eucalyptus*[16]) and the first formed pair of sepals and petals are generally larger than the other pair. Occasionally five sepals are formed. Apical growth of the sepal primordia soon ceases and further enlargement takes place either by zonal growth, in which the bases of the sepals are united to form a tube, or by separate intercalary meristems. In the former case a sepaline operculum is formed, while the latter results in chorisepaly, the length of the

Fig. 3. Flower bud of *E. kruseana* showing the free sepals (× 20)

Fig. 4. Flower bud of *E. guilfoylei*, seen from above to show the abscission scar of the sepaline operculum and the sutures of the petals, which form the petaline operculum (× 10)

sepals depending on the extent of intercalary growth. In all cases, at least the tips of the sepals remain free from each other and the tips of the petals are also free. Thus the tip of each operculum is traversed by a cuticle-lined canal. The limbs of the sepals may also be free from each other to an extent depending on the delay in initiation of the circular meristem at the base of the sepal primordia. In *E. pyriformis*, for example, the sepals are free from each other for about one-third their length (compare ref. 17), but die and are shed when the flower buds are about 1 cm. long. In a few species the sepals are free from each other but undergo abscission when the flower buds are less than 2 mm. long (for example, *E. brachyandra*, *E. raveretiana* and *E. microcorys*. The sepals are then either shed or, if they remain adherent to the petaline operculum, wither and are torn into fragments during its growth.

In certain species, for example, *E. microcorys*, *E. guilfoylei* (Fig. 4), *E. raveretiana* and *E. curtisii*, post-genital fusion of the margins of the petal primordia is delayed and the lines of fusion of the petals are clearly visible on the mature flower bud; in these species the petals are readily separable with a dissecting needle. In *E. erythrocorys*, in which the petals are all equal in size, the sutures are conspicuously thickened. In at least a few species of the Corymbosae (for example, *E. ficifolia* and, contrary to Willis[18], *E. gummifera*) the petals are not even post-genitally fused and are actually separate in the mature bud, although adherence to the sepaline operculum causes them to be shed with it as part of a single cap.

In immature flower buds the absence of a sepaline operculum may be surmised from the absence of the scar or line which, in species which lose the outer operculum precociously, usually indicates its former line of junction with the petaline operculum; but this criterion must be used with considerable caution. Certain species (for example, some of those in the Corymbosae) retain both opercula until maturity, and others may lose the sepaline operculum when the flower buds are still enclosed in the involucre and so small that no scar is later visible. In *E. lehmanni*, for example, it is necessary to examine buds no more than 1–2 mm. long in order to see the sepaline operculum, which has then ceased to grow. Each flower is sessile and grows through the outer operculum, leaving its disorganized remnants attached to the 'common receptacle' of the inflorescence. This species belongs in the series Cornutae, one of the more obviously natural groups[19], and related species also lose a sepaline operculum early in development, though none so precociously as *E. lehmanni*.

Also deceptive are the four-angled flowers of species such as *E. tetraptera*, which has been placed near the Eudesmieae on the supposition that the tips of the four angles of the calyx-tube represent free sepals. In fact, *E. tetraptera* loses a sepaline operculum in early development[20] and the 'tetrapterous' condition may be merely the result of compression of the young flower bud between its bracteoles (as in *E. globulus*, which loses its sepaline operculum relatively late, and *E. forrestiana*) and is, in any event, not a character common among chorisepalous species. *E. tetraptera* should be placed with the mallees (series Dumosae) which it resembles in its general habit, leaves and cotyledons and stamens.

Relatively few species retain both opercula until anthesis and they are placed in widely separated sections. Some of them comprise the series Corymbosae (bloodwoods) after the removal of the species grouped as the Clavigerae (Maiden) S. T. Blake[5] and, with the exception of *E. jacobsiana*, which is incorrectly placed by Blakely, of the yellow bloodwoods (*E. eximia*, *E. torelliana*, *E. peltata*, *E. bloxsomei*, *E. watsoniana*), all of which lose the sepaline operculum at an early stage in flower development. The yellow bloodwoods (in the restricted sense used above) should be grouped at least as a separate series, for which we suggest the name 'Eximieae'. It has already been suggested[5] that the Clavigerae may not be so closely related to the Corymbosae as has hitherto been thought, and the same suggestion may now be raised concerning the Eximieae. However, other characters (for example, the caoutchouc-like cuticle) peculiar to the bloodwoods (and found also in some species of *Angophora*) may indicate affinities despite the differences in behaviour of the sepaline operculum.

Certain species of the section Terminales also retain both opercula until anthesis. Many of them are placed in the series Siderophloiae which is, however, quite heterogeneous. Most of the species of the supposedly related Heterophloiae lose the sepaline operculum before the flowers reach maturity. The species in this section and in the heterogeneous section Porantheroideae–Normales are among the most 'difficult' in the genus and, as the mode of loss of the outer operculum (which we have found to be constant for a given species) can be readily determined from unopened buds on herbarium specimens, type specimens should be re-examined critically in order to clear up some of the taxonomic muddles. Due warning should be given, however, of the necessity of examining thin sections with the aid of the microscope.

In the majority of species with two opercula, the sepaline operculum is shed either at a very early stage in flower development or at least well before maturity of the bud. This group includes Transversae, Cornutae and Subcornutae, Dumosae and

Exsertae, which are all fairly natural series of the section Macrantherae. It also includes sections Macrantherae–Normales, Graciles, Micrantherae and Platyantherae, a large and heterogeneous group. It is here that the main problems of *Eucalyptus* taxonomy now lie, and further criteria are required to elucidate relationships. To this end we have collected a considerable amount of information on the presence or absence of oil glands in the filaments and of supernumerary glands on the connective or anther lobes, characters which have either not been recorded or not deemed worthy of more than casual reference. In the Cornutae and Dumosae, for example, the filaments have oil glands and there are supernumerary glands on the connective or anther lobes of many of the species of these series, which are probably quite closely related to each other.

While the nature and behaviour of the perianth in *Eucalyptus* remained obscure, comparisons with supposedly distinct but related genera, such as *Angophora*, *Pleurocalyptus* and *Cleistocalyx* were difficult to make and the distinctions between *Eucalyptus* and these genera seemed clear. Both *Cleistocalyx* (*Acicalyptus*) and *Pleurocalyptus* have a sepaline operculum and free petals, which are adherent and are shed together as a cap. This cap often remains attached to one side of the open flower bud. The petals are fringed and highly glandular. All these characteristics are shown by certain members of the Corymbosae. The ovary of *Pleurocalyptus* is semi-superior, as is the ovary in certain species of *Eucalyptus*, for example, *E. deglupta*, *E. raveretiana*. Again, the similarities between the vegetative characters of *Angophora* and those of some species of *Eucalyptus* are striking; the resemblance between *E. jacobsiana* and *E. setosa* and certain species of *Angophora* is remarkable. It is tempting to suppose that *Eucalyptus* has had a polyphyletic origin and that *Monocalyptus*, in particular, has developed quite independently of the rest of the genus.

We wish to thank the Government botanists of Victoria and New South Wales for allowing us access to the State Herbaria. Our thanks are due also to many foresters in Victoria and colleagues at Melbourne for collecting specimens, and to Mr. E. A. Matthaei and Mr. R. Oldfield for assistance with microscopy and photography.

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VARIOUS TYPES OF ACQUIRED RESISTANCE TO EXPERIMENTAL 'ALLERGIC' ENCEPHALOMYELITIS

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IN our previous communications[1,2] two facts of acquired resistance to experimental allergic encephalomyelitis have been reported, namely, (*a*) after recovery from the conditions and (*b*) after immunization with a mixture of the non-encephalitogenic brain of an embryo or new-born rabbit with the adjuvant.

Prior to our work, three other facts of induced resistance to experimental allergic encephalomyelitis have been described: (1) Ferraro and Cazullo[3] inoculated a mixture of monkey brain with paraffin oil and lanolin (without mycobacteria) in 15 daily doses to guinea pigs. On the fourth day after the last inoculation, the animals were treated with the encephalitogenic mixture. Out of 12 animals, 1 contracted the disease compared with 12 or 13 in the control. (2) Condie *et al.*[4] reported resistance in rabbits 45 days after immunization with homologous spinal cord. (3) Lumsden[5] reported resistance after inoculation with non-encephalitogenic mixtures of an alcohol extract of the brain or adrenals with the adjuvant and suggested that the resistance was called forth by the adjuvant.

Following our publication, Kies and Alvord[6] confirmed Lumsden's suggestion. These workers suggest that in our experiments acquired resistance has been induced by the adjuvant, in particular, by the killed tubercle bacilli.

Our evidence confirms that immunization with the adjuvant alone can indeed account for a certain amount of resistance to experimental allergic encephalomyelitis, but as regards its morphology and mechanism it is to be distinguished from the resistance acquired after recovery from the condition or after immunization with a mixture of embryo brain and the adjuvant.

Experimental proof is adduced below that there are various types of acquired resistance to experimental allergic encephalomyelitis. These types of resistance can be understood from consideration of that acquired by animals after recovery from experimental allergic encephalomyelitis, which makes doubtful the allergic nature of the condition itself. Prior to our experiments, this fact has not been described in the literature,

in which even quite opposite results can be found[6].

In the following experiments a somewhat modified technique from that previously described[1,2] has been used: a total challenge dose of 1 ml. of the encephalitogenic mixture was injected subcutaneously to guinea pigs simultaneously in 5 loci in doses of 0·2 ml. In most of the experiments large animals (500–1,000 gm.) were used, since they have been found to be particularly susceptible to experimental allergic encephalomyelitis.

In the first experiment, 53 guinea pigs were repeatedly treated 1–9 months after recovery from experimental allergic encephalomyelitis with the encephalitogenic mixture. Only one case of disease was recorded as against 78–100 per cent in the controls. The acquired resistance following recovery could not be transmitted passively via the blood and the cells of the lymphatic nodes and spleen[8].

In the next series of experiments guinea pigs were immunized two to three times with various ingredients, and after 3–6 weeks were challenged with the encephalitogenic mixture.

Guinea pigs immunized with a mixture of the brain of new-born rabbits with the adjuvant proved resistant to experimental allergic encephalomyelitis. The disease incidence was 0/31 in the experiment and 32/37 in the control (86 per cent). (Here and below the denominator indicates the number of animals in the experiment, and the numerator the number of individuals contracting the disease.) After challenge, in the cerebrum of the animals with resistance acquired after recovery from experimental allergic encephalomyelitis and after immunization with a mixture of brain of new-born rabbits with adjuvant there was found the pathomorphological picture of experimental allergic encephalomyelitis without demyelinization along with complete clinical health.

The disease incidence among the guinea pigs immunized with the adjuvant alone varies from 0/31 to 4/9 (44 per cent) compared with 32/37 (86 per cent) and 14/18 (77 per cent) in the controls. However, in the central nervous system of guinea pigs which have acquired resistance through immunization with the adjuvant alone, no changes have been noted either prior to challenge or after it. This resistance apparently does not involve the central nervous system, being merely 'peripheral'. This is a resistance to the effect of the adjuvant or, more precisely, to the peripheral mechanisms of induction of experimental allergic encephalomyelitis but not to experimental allergic encephalomyelitis itself which develops in the central nervous system.

No resistance has been achieved through immunization with a 50 per cent suspension of the brain of new-born rabbits *per se* or with lanolin and paraffin oil, or with large doses of killed B.C.G. *per se* (200 mgm. doses twice in 5 loci) or with lanolin and paraffin oil.

Daily injections for 15 days of 0·2 ml. of a mixture of adult rabbit brain with lanolin and paraffin oil (Ferraro and Cazullo's method[3]) ensured resistance to the administration of the encephalitogenic mixture on the fourth day after completion of the injections, namely, 2/10 (20 per cent) in the experiment as compared with 7/9 (77 per cent) in the control.

The next experimental series suggests the existence of acquired resistance to experimental allergic encephalomyelitis of the interference type. Guinea pigs were injected daily for 16 days intraperitoneally or subcutaneously with 3 ml. of a 20 per cent cerebral suspension of adult rabbits without adjuvant. Between the sixth and seventh injections they were treated with an encephalitogenic mixture. The disease incidence in the experiment was 3/16 (18 per cent) and 8/11 (72 per cent) in the control. No interference effect was observed on inoculation according to the same scheme: (1) with the brain of new-born rabbits; (2) with large doses of killed B.C.G. (16 doses of 250 mgm.); (3) with lanolin and paraffin oil. We could not confirm our suggestion[1,2] as to the presence in the brain of new-born rabbits of a factor responsible for the development of resistance to experimental allergic encephalomyelitis and different from the encephalitogenic factor.

We could not distinguish the antigens of rabbit embryo brain from the brain of adult rabbits in the anaphylaxis reaction with desensitization, using Zilber's method[8,9].

Multiple daily subcutaneous and intraperitoneal injections of large doses of adult rabbit brain also prevent the contraction of experimental allergic encephalomyelitis when they are started simultaneously with the administration of the encephalitogenic mixture.

Moreover, daily 20 intraperitoneal injections of 5 ml. of 25 per cent adult brain suspension, started 5 days after the injection of the encephalitogenic mixture, completely prevent experimental allergic encephalomyelitis (0/13 in the experimental group as compared with 12/15 in the control one). Even if treatment with massive doses of the cerebral suspensions started at a later date, it checked the disease.

In some experiments single or two-fold intracerebral inoculation of brain emulsion of adult rabbits interfered with the effects of the simultaneous or previous administration of the encephalitogenic mixture.

The following types of acquired resistance to experimental allergic encephalomyelitis can be tentatively distinguished: (1) acquired resistance of the central type (recovery after the disease, immunization with the brain of new-born rabbits with adjuvant); (2) resistance of the interference type (administration of the cerebral substance shortly before or after administration of encephalitogenic mixture); (3) resistance of the peripheral type (administration of the Freund adjuvant).

We have found recently in the progeny of guinea pigs treated during pregnancy some signs of experimental allergic encephalomyelitis[8–10]. This progeny at the age of five months had evidenced some degree of resistance of the disease.

We acknowledge the technical assistance of I. S. Keeselõva and E. A. Kanigina.

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FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, December 14

Institution of Electrical Engineers (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Why Hi-Fi?", opened by Mr. P. P. Eckersley.

Society of Chemical Industry, Pesticides Group (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Mr. P. B. Cornwell: "Insect Control by Gamma Irradiation".

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 8.30 p.m.—Baron G. de Gerlache de Gomery: "The Belgian Antarctic Expedition".

Tuesday, December 15

Institute of Marine Engineers (at the Memorial Building, 76 Mark Lane, London, E.C.3), at 5.30 p.m.—Mr. K. Maddocks: "Some Aspects of Marine Reactor Security".

Institute of Physics (at 47 Belgrave Square, London, S.W.1), at 5.30 p.m.—Mr. K. G. Freeman: "Tubes for Colour Television".

Institution of Electrical Engineers, Measurement and Control Section (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Data Handling Problems in Atomic Installations", opened by Dr. D. Taylor.

Wednesday, December 16

Powder Metallurgy Joint Group of the Iron and Steel Institute and the Institute of Metals (in the Hoare Memorial Hall, Church House, Great Smith Street, London, S.W.1), at 9.30 a.m.—Symposium on "The Powder Metallurgy of Magnetic Materials".

Royal Meteorological Society (at 49 Cromwell Road, London, S.W.7), at 5 p.m.—Dr. H. Wexler: "Seasonal and other Temperature Changes in the Antarctic Atmosphere"; Mr. W. Hitschfeld: "Patterns of Hailstorms in Alberta".

Royal Statistical Society (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.15 p.m.—Sir Hugh Beaver, K.B.E.: "Statistics as a Tool of Management" (Presidential Address).

Institute of Petroleum (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. A. C. Hartley: "Pipelines".

Institution of Mechanical Engineers (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Dr. S. G. Hooker: "The Future of Air Breathing Engines in Aviation" (James Clayton Lecture).

Society of Chemical Industry, Corrosion Group (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Discussion of Research Papers recently published by Members of the Group.

Society of Instrument Technology (at Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Mr. J. F. Coales: "Random Inputs and Load Variations".

Thursday, December 17

British Computer Society, London Branch (at the Northampton College of Advanced Technology, St. John Street, London, E.C.1), at 2.30 p.m.—Discussion on "Conversion between Analogue and Digital Representation".

Society of Chemical Industry, Road and Building Materials Group (at 14 Belgrave Square, London, S.W.1), at 2.30 p.m. and 5.30 p.m.—Scientific Papers.

Institution of Mining and Metallurgy (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. A. J. Robinson: "Relationship Between Particle Size and Collector Concentration"; Mr. D. Borchers: "Deepening of No. 1 Vertical Shaft at Premier (Transvaal) Diamond Mining Co., Ltd.".

London Mathematical Society (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Prof. E. V. Schenkman: "Supersolvable Groups".

Institution of Electrical Engineers, Education Discussion Circle (at Savoy Place, London, W.C.2), at 6 p.m.—Discussion on "The Presentation of the History and Philosophy of Science", opened by Prof. H. Dingle.

Thursday, December 17—Friday, December 18

Physical Society (in the Townsend Lecture Theatre, The Clarendon Laboratory, Oxford), at 10 a.m. daily—Conference on "Electron-Phonon Interaction in Solids".

Friday, December 18

Society of Chemical Industry, Fine Chemicals Group (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. F. E. Camps: "Some Aspects of Forensic Science".

Friday, December 18—Saturday, December 19

British Psychological Society (at University College, Gower Street, London, W.C.1)—Conference.

Saturday, December 19

Biochemical Society (in the Department of Chemical Pathology, King's College Hospital Medical School, Denmark Hill, London, S.E.5), at 10.30 a.m.—389th Meeting.

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Assistant Lecturer or Lecturer in Physics—The Secretary, The University, Exeter (December 19).

Assistant Lecturer or Lecturer in the Department of Zoology—The Registrar, The University, Liverpool (December 19).

Shepherd Research Fellow in the Department of Pathology, for work on immunological problems of ocular disease—The Director, Department of Pathology, Institute of Ophthalmology (University of London), Judd Street, London, W.C.1 (December 31).

Scientific Officer (with a good honours degree in botany and experience in plant physiological research) in the Botany Department, to work on physiology of growth and yield of the sugar beet, particularly on the causes of variation between places and seasons in field crops—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (January 1).

Assistant Lecturer or Lecturer in Physics—The Registrar, University College of South Wales and Monmouthshire, Cathay's Park, Cardiff (January 2).

Assistant Lecturer or Lecturer (with an honours degree or equivalent, and preferably experience in hydraulics) in the Department of Fluid Mechanics—The Registrar, The University, Liverpool (January 4).

Lecturer in Biblical Archæology at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, January 4).

Research Fellows (2) in the Department of Geography, Research School of Pacific Studies, Australian National University, Canberra—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, January 4).

Research Fellow (with a good knowledge of electronics) in the Department of Physics, to engage in a research programme on spark discharges, in association with Prof. J. M. Somerville—The Registrar, University of New England, Armidale, N.S.W., Australia, or The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (January 9).

Chair of Physiology in the University of Malaya (Singapore Division)—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (January 15).

Lecturer or Assistant Lecturer in Pharmaceutics at the University of Malaya (Singapore Division)—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (January 15).

Chair of Chemistry—The Registrar, University College of Wales, Aberystwyth (January 16).

Lecturer or Assistant Lecturer in Physiology—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (January 16).

Lecturer/Senior Lecturer in Mechanical Engineering at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, February 2).

Assistant Director of the Meteorological Department, Ghana—The Director of Recruitment, Ghana High Commissioner's Office, 13 Belgrave Square, London, S.W.1.

Assistant Lecturer and Demonstrator in Physics—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8.

Irrigation Officer (with a university degree in agriculture, or a science degree with agricultural experience, and training or experience in soil physics or irrigation) in Swaziland, to carry out irrigation experiments on sugar cane, citrus and other crops, and to take charge of a new experimental station—The Director of Recruitment, Colonial Office, London, S.W.1, quoting Ref. BCD.63/76/014/C1.

Research Assistant (with an honours degree in electrical engineering or physics) in the Department of Engineering, for an investigation of certain aspects of the high current vacuum arc—The Secretary, The University, Old College, South Bridge, Edinburgh.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

University of Birmingham. Report of an Enquiry into the Suitability of the General Certificate of Education Advanced Level Syllabuses in Science as a Preparation for Direct Entry into First Degree Courses in the Faculty of Science. Pp. v+212. (Birmingham: The University, 1959.) [221

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LETTERS TO THE EDITORS

ASTRONOMY

A Search for Neutral Atomic Hydrogen in Globular Clusters

GLOBULAR clusters can be expected to contain interstellar matter if, as is currently believed, all stars in their evolutionary processes end up as white dwarfs. Theory predicts that a star evolving to the white dwarf stage will shed material if its initial mass is greater than about $1{\cdot}2\ M\odot$, where $M\odot$ is the mass of the sun. Loss of mass need not stop when the star reaches this limit. That it may indeed continue is suggested by the low mean mass of about $0{\cdot}6\ M\odot$ found for white dwarfs[1]. In a globular cluster, the amount of matter thus ejected from evolving stars will be a significant fraction of the total mass of the cluster. However, it is likely that during the lifetime of the cluster most of this matter will be removed by (1) passages through the plane of the galaxy which would sweep out the dust and gas, and (2) presumably by the establishment of equipartition of energy between the stars and intraglobular matter.

During a time equal to the period of oscillation of the cluster through the galactic plane, $\sim 10^8$ years, the stars in a globular cluster will shed mass of the order of $10^2 M\odot$. This estimate follows from the above value for the mean mass of white dwarfs and is based on the luminosity function for M 3. If this ejected material were to collect into clouds the second process listed above would be made very ineffective for the removal of matter from clusters. Such clouds may exist in clusters. Many clusters show dark regions and lanes in the projected stellar distribution. A study of a mathematical model of a globular cluster shows that in general these lanes are too large to be attributed to statistical fluctuations in the star distribution. M 13 contains an outstanding example of such a dark region; knowledge of such lanes is at least 100 years old[2]. Although these obscured regions may be foreground clouds, the frequency with which they are found 'in' clusters makes it unlikely that they are all external to the clusters.

The material thus shed from evolving stars and from which the clouds form can be expected to have hydrogen as its principal constituent. If unionized and atomic, such hydrogen should be detectable. An attempt was made to measure the 21-cm. emission of two globular clusters, M 3 and M 13, with the 85-ft. telescope of the National Radio Astronomy Observatory. From 30 to 37 scans in right ascension centred at the declinations of the clusters were made at the frequency of the hydrogen line corrected for the observed optical radial velocities. Although there is a relatively large probable error in these velocities, it is likely they would fall within the 200 kc./sec. band-width (42 km./sec.) used for these observations. The equipment was calibrated by observing Virgo A, using Westerhout's[3] value for the antenna temperature. The results are shown in Figs. 1 and 2. The mean values of the antenna temperature, ΔT_A, and the probable error of these means is shown. The half-power beam-width and the optical centre of the clusters are indicated at the bottom of each figure. The optical diameters of both

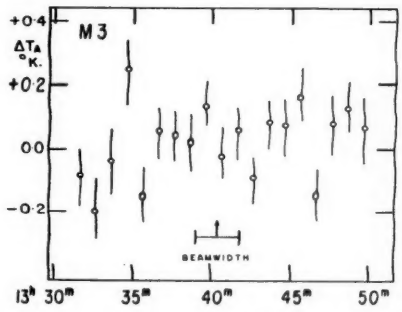

Fig. 1. 21-cm. antenna temperature in the region of the globular cluster M 3. The arrow indicates the optical centre

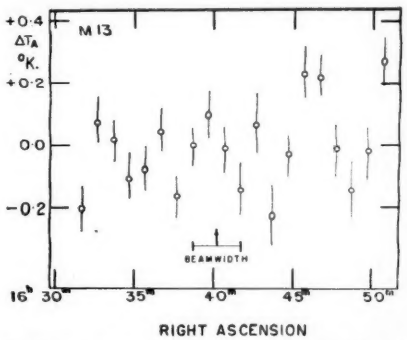

Fig. 2. 21-cm. antenna temperature in the region of the globular cluster M 13. The arrow indicates the optical centre

clusters are smaller than the half-power beam-width.

A conservative upper limit for ΔT_A for both M 3 and M 13 is 0·4°K. An upper limit to the mass of neutral hydrogen may be computed from this antenna temperature[4]. For a source smaller than the half-power beam-width and for $\tau \ll 1$, we have:

$$M_{\mathrm{HI}}/M\odot = 1{\cdot}3 \times 10^{-6}\ \Delta T_A\ \Delta V d^2$$

Here ΔT_A is the maximum antenna temperature, ΔV the total line width to half-intensity in km./sec. and d is the distance of the object in parsecs. ΔV is not available from these observations; however, it must be less than 20 km./sec. since the escape velocity from a globular cluster is of the order of 10 km./sec. A value of $\Delta V = 7$ km./sec. was adopted. Distances for the clusters are from Arp[5]. Values for the upper limits of the mass of ionized hydrogen are 700 $M\odot$ for M 3 and 200 $M\odot$ for M 13. It should be noted that M 3 is approximately twice as massive as M 13 if we assume a constant ratio of mass to luminosity for globular clusters.

For some of the dark lanes in globular clusters the optical opacity is high. The computed upper limits of the mass of neutral hydrogen may therefore be incorrect for the following reasons:

(1) The opacity at 21 cm. may not be small. For a specified gas kinetic temperature, increasing τ results in increasing ΔT_A.

(2) The gas kinetic temperature may be quite low. Such a low temperature could be due to both the

high optical opacity and a lower radiation temperature for intraglobular space.

(3) A significant amount of neutral hydrogen may be present but in a molecular state and therefore undetectable.

Intraglobular matter in an amount larger than the observed upper limit of neutral hydrogen is predicted. This together with the apparent existence of absorbing lanes in globular clusters implies that (2) and/or (3) above will explain this discrepancy. It should be noted that the formation of molecular hydrogen would be favoured in cool, optically dense clouds. A detailed discussion of intraglobular clouds and their effects on the stellar population of a cluster is being prepared for publication.

The observations described above were made while I was a guest investigator at the National Radio Astronomy Observatory as a National Science Foundation postdoctoral fellow.

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Sept. 10.

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Volcanoes on the Moon

The recent observations by Kozyrev[1] of phenomena on the surface of the Moon, which he ascribed to volcanic activity, have stimulated a considerable amount of conjecture. Fremlin[2] has put forward an hypothesis based on radioactive heating from the interior and an assumed very low thermal conductivity of the lunar surface. This model leads to the prediction of sub-surface temperatures high enough to melt basaltic rocks. Objections to this hypothesis have been pointed out by Jaeger[3].

An alternative hypothesis which offers an explanation of the observations, and also enables several predictions to be made which should be amenable to observational test, can be based on the assumption that there exists on the lunar surface dust composed of radiation-damaged material containing free radicals and reactive molecules. This suggestion has also been advanced by Platt[4] to explain the colour of the surface of the Moon and its generally low albedo. A similar proposal has been made by Donn and Urey[5] to explain cometary outbursts.

The basic assumption is now made that chemically unstable materials of this nature may be triggered into exothermic reaction by the arrival of an unusually intense burst of high-energy particles or photons. This leads to the suggestion that the emission of gases observed by Kozyrev be ascribed to an explosively exothermic recombination in unstable radiation-damaged material. The triggering agency responsible could be an abnormally high level of incident radiation, photonic or corpuscular, as might occur during or following periods of intense solar activity. The present hypothesis rests on two assumptions: (*a*) that the upper layer of the surface of the Moon is of such a composition that it is capable of storing energy chemically in the form of free radicals and reactive chemical species; (*b*) that the temperature of this material is low enough that, even during the lunar day, in the absence of disturbances of the kind postulated, no chemical reaction takes place.

Little is known about the chemical composition of the lunar surface, but measurements of the surface temperature have been made[6,7]. Daytime temperatures up to 100°C. have been found from measurements with a vacuum thermocouple. Similar estimates are obtained from microwave observations[8]. However, as Baldwin has emphasized, and as implied by the measurements of Pettit and Nicholson, one must be careful in assigning a unique temperature to any particular place on an airless body, because the surface irregularities will lead to large departures from the observed average value; much evidence points to the Moon having a very rough surface on the small scale. Although the average measured temperature at the subsolar point exceeds 100°C., "a rock placed in a shadow would soon drop over 200°C. in temperature"[7].

If, as suggested recently by Fielder[9] in seeking an explanation for the low general albedo of the Moon, the lunar surface is highly vesicular in nature, the shade temperature inside such vesicles may well be −100°C. or lower even under a noonday sun. A mechanism by which such vesicles might have been formed will be discussed by me elsewhere.

Free radicals of low molecular weight could not exist at temperatures much above 200°K. Because of the very low ambient pressure, the same applies to solids composed of volatile, reactive molecules such as H_2O_2, C_2H_2 and C_2H_4, postulated by Donn and Urey to exist in comets. However, such species could exist within shaded vesicular hollows as described above. Furthermore, such radicals and reactive molecules could be produced in these vesicles over long periods by the incidence of high-energy particles and by the energy of high-energy photons scattered from the walls into the hollow.

There remains the question of the possibility of storing energy by radiation damage to essentially non-volatile rocky material. As yet little is known about this, but experiments on γ-irradiation of terrestrial rock samples could yield an answer. Finally, it is worth pointing out that should the surface be covered with very finely divided dust, produced by thermal erosion, this in itself represents a mechanism for energy storage. Once started, a process of nucleation and consolidation might proceed exothermically.

The present model, based on the two basic assumptions discussed above, immediately leads to several implications and predictions:

(1) A correlation may exist between enhanced solar activity and lunar 'volcanoes'. Such periods of solar disturbance are accompanied by increased emission of short-wave ultra-violet radiation, X-rays and the solar component of cosmic rays. These reach the Earth in eight minutes for the photonic radiation and within half an hour for the cosmic rays. In addition to all these, geomagnetic storms sometimes occur at an average time of 30 hr. after the solar disturbance. These are attributed to the arrival of a jet of slower-moving corpuscles ejected from the active area on the Sun. It is apparently a matter of chance whether or not the Earth happens to be in the cone of emission at the proper time to intercept the stream. Also the energy of these particles is very much less than that of the solar cosmic rays. Any correlation between solar activity and lunar 'volcanoes' should, therefore, be sought immediately following the period of solar disturbance when high-energy photons or cosmic rays could set off an explosive reaction on the lunar surface. Kozyrev's first observation of Alphonsus was made at 0400 Moscow time (0100 U.T.)[1b] when he noticed an

'unusual reddish hue of the central peak'[1a]. Later, at 0630 Moscow time, after making a second spectrogram, he observed a sudden decrease in intensity of the unusually bright central peak. Solar flare data[10] record a flare on this date which commenced at 0049, reached its maximum activity at 0055 and ended at 0121. The correspondence between the beginning of this flare and Kozyrev's first observation 11 min. later is very good. It would seem to be well worth while to observe the Moon more intently immediately following periods of increased solar activity.

(2) Lunar 'volcanoes' should be observed always on the sunlit portion of the surface of the Moon and never in the dark portion. The crater of Alphonsus observed by Kozyrev was in fact in sunlight at the time.

(3) Areas close to the equator are more favourably situated to receive the maximum dose of radiation. Alphonsus is situated on the meridan and close to the centre of the lunar disk.

(4) If reactive molecules play a part, spectroscopic evidence for free radicals might be expected. Kozyrev did in fact observe in his second spectrogram bands attributed to C_2 and C_3.

(5) Finally, if the present hypothesis be true, 'volcanic' activity is not necessarily restricted to craters, but eruptions and emissions of gas might be expected at any point on the surface.

On the basis of the present ideas, Kozyrev's observation is not incompatible with a meteoric hypothesis for the formation of the craters of the Moon.

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METEOROLOGY

Oxygen Isotope Variations in Antarctic Snow Samples

THE relative abundance of oxygen isotopes in atmospheric precipitation being closely related to temperature, it has been suggested that the variations of the ratio oxygen-18/oxygen-16 in snow should be used in glaciological studies of polar regions[1,2]. It has been recently announced that very promising results have been already obtained by analysing cores of old ice from deep bore holes in Greenland[3].

We wish to report here on an examination of the isotopic composition of oxygen in samples of recent snow, collected in known meteorological conditions on the coast of Antarctica.

The ratio oxygen-18/oxygen-16 has been measured in samples of snow collected during the International Geophysical Year at the King Baudouin Base (Belgian Antarctic Expedition 1957–1958 under the leadership of Gaston de Gerlache). The base is located on the Princess Ragnhild Coast at about 15 km. from the coast, at lat. 70° 26' S. and long. 24° 19' E.

Samples of precipitated snow, drift-snow and rime deposits were collected during the period extending from April, 1958, until February, 1959.

The mass-spectrometric analyses were carried out in Pisa (Laboratory of Nuclear Geology). Experimental procedures used in the measurement of the oxygen-18/oxygen-16 ratio are essentially the same as described by Epstein and Mayeda[4]. They involve equilibration of carbon dioxide of known isotopic composition with the water sample and analysis of the equilibrated carbon dioxide in a double collector mass-spectrometer of the MacKinney–Nier type.

The results reported here are corrected for the various factors discussed by Craig.[5] They are expressed in terms of relative deviation per mille defined as follows:

$$\delta = \frac{(^{18}O/^{16}O)\ \text{sample} - (^{18}O/^{16}O)\ \text{standard}}{(^{18}O/^{16}O)\ \text{standard}} \times 1{,}000$$

Table 1

Date (1958–1959)		T° C.	δ‰
14–15/4	*P*	− 8	−16·2
28/4	*S*	−10	−19·2
21–22/5	*P*	− 9	−24·6
23/5	*P*	− 8	−28·7
18/6	*R*	−20	−28·3
19/6	*R*	−26	−29·5
20/6	*R*	−20	−32·2
3/7	*P*	−10	−25·9
19/7	*S*	−18	−30·8
22/7	*S*	−20	−28·5
12/8	*S*	−24	−35·8
17/8	*P*	−19	−27·0
18/8	*R*	−23	−31·7
20/8	*R*	−30	−29·9
23/8	*P*	−23	−26·3
27/8	*S*	−30	−28·7
29/8	*P*	−37	−32·6
8/9	*P*	−18	−28·1
23–24/9	*S*	−15	−29·1
28–29/9	*S*	−18	−28·3
5/10	*P*	−12	−24·6
19/10	*P*	−15	−26·7
1/11	*P*	− 8	−21·4
11/11	*P*	−10	−18·4
17/11	*P*	− 5	−15·7
28/1	*P*	− 4	−14·6
31/1	*R*	−15	−19·3

P, precipitation; *S*, drift-snow; *R*, rime

The values for δ given in Table 1, column 4, are expressed relative to the Chicago Standard PDBI, which is practically equivalent to average ocean water. Column 3 shows the average ground temperature during the sampling period. The complete meteorological data are, however, available from radio soundings taken twice daily. They will be published elsewhere in a more extensive paper.

The following conclusions can be drawn from the results shown in Table 1:

(1) The average value of δ is about −25. This value is in good agreement with the multiple-stage distillation model proposed by Epstein and Mayeda[4] and developed by Dansgaard[1]. For comparison, we quote the following average values of δ found in samples of various origin by these authors:

Ocean water : 0
Precipitation in temperate climate : − 10
Precipitation in polar tundra climate (South Argentine, Greenland coast) : − 16
Greenland ice cap : −30 to −35
Antarctic ice-cap at South Pole : −50.

(2) A strong seasonal variation of δ is obvious, the summer samples being richer in oxygen-18 than the winter samples. The largest variation of δ in precipitated snow is $\Delta\ \delta = 16$, corresponding to an observed

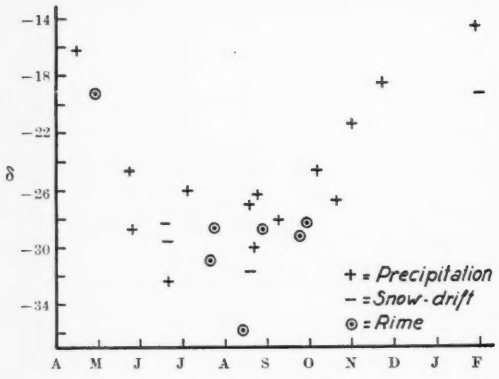

Fig. 1

temperature variation of about 20° C. Such dependance on temperature is in reasonable agreement with the formulæ developed by Dansgaard on the basis of the Raleigh distillation formula.

The observed seasonal variation of δ would be accounted for by the seasonal variation in the range of cooling of an air mass saturated with oceanic water vapour. It is worth noting that introduction of a large amount of water vapour from the Antarctic Continent should lead to the reverse effect, that is to say, an increase of ratio of oxygen-18 to oxygen-16 in summer samples, because evaporation from the ice-cap is predominant during summer-time.

(3) The ratio oxygen-18/oxygen-16 for snow-drifts seem to follow the same seasonal trend as that for precipitated snow. This is a strong indication that snow-drifts are formed from recently precipitated snow and not from an average mixed source.

It is also interesting that the ratio oxygen-18/oxygen-16 in drift snow is not systematically lower than in precipitation, which shows that snow-drifts do not come from very far south, where, owing to higher altitude and colder climate, one would expect lower values of δ.

It is planned to measure the tritium and deuterium content of the same samples. This would give a more complete picture of their origin and their evolution.

We believe that stable isotopes are promising tools for the investigation of many important problems in the meteorology and glaciology of the Antarctic, and it would be worth while to give more attention to such work in the programmes of antarctic expeditions.

We are indebted to Capt. X. de Maere, chief meteorologist of the Belgian Expedition, and to Prof. E. Tongiorgi, director of the Laboratory of Nucelar Geology, University of Pisa, for helpful discussions.

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RADIOPHYSICS
Inadequacy of Scatter Mechanisms in Tropospheric Radio Propagation

For a decade or more the propagation of radio waves far beyond the horizon in the very high-frequency, ultra high-frequency and *super* high-frequency bands, together with their characteristic fast and deep fading, has been explained almost exclusively in terms of temporally and spatially random scattering from dielectric irregularities in the atmosphere. The scattering was assumed to occur at 'blobs' located in the region of intersection of transmitter and receiver antenna lobes, and some agreement with experiment was found to be possible by arbitrary adjustment of boundary conditions and statistical parameters.

In recent years, as important observations have remained unexplained by the scatter theories, doubts of its validity have increased; and Watterman's[1] beam-swing tests have now weakened one of the strongest arguments supporting scatter (the loss of antenna gain).

It is a necessary consequence of any scatter theory that the propagated field should be randomly distributed in space as well as time far beyond the horizon. Whilst the randomness in time is well known and easily observed, no attempts appear to have been made to examine the spatial distribution in this respect. Ideally it is required to map instantaneous field strengths both vertically and horizontally, but such a task is clearly prohibitive. A study must therefore be made of signals received from moving sources or by moving receivers.

Most of the published data and a number of unpublished reports available have been carefully scrutinised and evaluated for information on the spatial field distribution, and a remarkable regularity in the field strength versus distance characteristics has been revealed. Results which are considered as conclusive were obtained especially from sea-runs, where the uniformity of the terrain is preserved, especially when the continuous records were averaged over about ten minutes.

Fig. 1 shows an estimated characteristic for a test at 200 Mc./s. described by Kitchen, Richards, and Richmond[2], the points indicating their ten-minute averages. The evidence of a coherent field far beyond the horizon is strong, and these results are typical of the large number studied.

Further results taken on the same run nine months later show a similar characteristic, and corresponding lobes on the two sets of results can be easily identified. Thus it appears that the spatial field distribution is generally non-random, and is represented by a com-

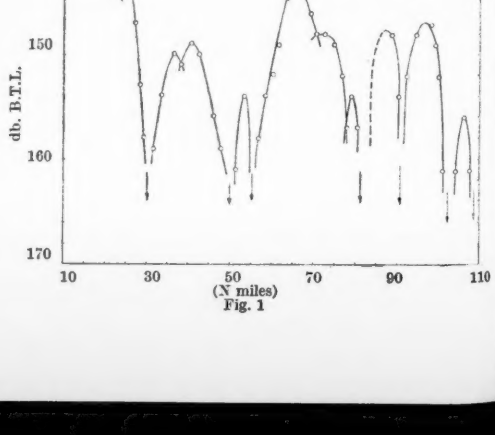

Fig. 1

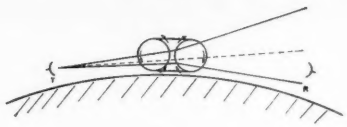

Fig. 2

plex interference pattern arising from multipath propagation. As a result of fluctuations in the bending of the field over the horizon, the maxima and minima can move relative to a receiver and cause the familiar fading associated with tropospheric propagation.

The conclusion that a scatter mechanism is not principally responsible for tropospheric propagation leads to the question of a replacement theory. I developed in May 1958 an unpublished theory which shows that the main features of tropospheric propagation can be adequately explained both qualitatively and quantitatively in terms of toroidal thermal motions in the atmosphere which are known as 'Benard cells'[3].

A toroid arising from the turbulent state of the atmosphere is depicted in Fig. 2. The systematic rising of warm air in the 'eye' of the toroid causes small but important changes in refractivity so that the toroid acts as a weak lens. Such a lens gives rise to a horizontal contraction and vertical divergence that can account for the propagation beyond the horizon. The lenses have an average size of a few kilometres diameter, and their movement together with their creation and decay accounts adequately for the main fading characteristics.

It can be shown that the convection heat currents due to these toroids are normally in a state of saturation, and as a consequence of this the lapse rate and mean refractive index gradient do not vary strongly with the diurnal cycle.

The details of both the analysis of experimental data and the toroid theory are being prepared for publication. The conclusion is that the spatial field distribution is not random, the field being essentially coherent, and the fluctuations at a fixed position are due to shrinkage and expansion of the interference pattern with atmospheric changes.

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PHYSICS

Atomic Units

Comparisons between the results of accurate quantum-mechanical calculations on atoms and molecules have been made unnecessarily complicated and sometimes even impossible by the lack of an agreed and unambiguous system of units. A calculated binding energy, for example, may well be reported in kcal./mol. or eV. on one hand, or in Rydbergs or Hartree atomic units (double Rydbergs) on the other. The latter pair have the advantage of being the primary result of the calculation but the disadvantage of being frequently denoted by the same cumbersome symbol a.u. Furthermore, the same symbol is also used for a unit of length (the first Bohr radius) for a unit of electric moment and, in quantum field theory, for a quite different energy. To compound the confusion, some authors have even used double Hartree a.u. (quadruple Rydbergs). Then, in addition, atomic units usually leave unspecified whether or not a *reduced* mass is given unit value instead of the electron mass, or indeed whether other modifications have been made (see below). Even when the modification of one unit is specified, its effect on the other units may still be quite uncertain until it is agreed which units are basic and which are derived from them.

The use of such more conventional energy units as kcal./mol., eV., or cm.$^{-1}$, for example, avoids some of the confusion concerning multiplicity of unit, but has other decided disadvantages. These units are not the primary result of the calculation and the conversion factors depend on the currently accepted best values of $\hbar$, m, e, c, N, etc. The change in these during the past thirty years has already been sufficient to cause considerable difficulties in comparing accurate calculations done in different years.

It seems to us that the time is ripe for some standardization in the use of units, for some agreed notations for the most common units and for a general agreement to specify units so carefully in writing papers that future scientific generations may be spared such difficulties.

In particular we would like to recommend that the results of quantum mechanical calculations be reported always in the original primary units. If the results are desired in such secondary units as kcal./mol. or eV., these should be given in addition to the primary result. Furthermore, the conversion factor used should be stated explicitly.

To specify a system of atomic units it is necessary to name three quantities as basic and derive the remainder from them. There seem to us to be good reasons for continuing the convention wherein these three are taken as e, $\hbar$, m and the units of energy, length, etc., derived from them. This convention is, of course, quite independent of the problem of calculating the appropriate conversion factors for the various units. It may be more expedient to regard the energy conversion factor as a basic one when these have to be found from experiment.

There seems to be an increasing tendency to adopt the Hartree system of atomic units[1]. We would like to recommend that it be adopted as widely as possible in the future. Since the energy unit in this system is the one most generally needed, it seems appropriate that its name be simplified to the 'Hartree' and denoted by H. Thus we define :

$$H = me^4/\hbar^2$$

The conversion factor from Hartrees to cm.$^{-1}$ is then precisely twice as large as the infinite Rydberg, R_∞. In H we imply either that m is the electronic mass or that the accuracy of the calculation is such that nothing is lost by making this assumption.

In many calculations it becomes convenient to use as unit mass some appropriate reduced mass :

$$\mu = mM/(m+M)$$

where m is the electronic mass. Since the energy is a derived unit, it is changed by this and we suggest that the corresponding energy be the 'reduced Hartree' and be denoted by H_R :

$$H_R = \mu e^4/\hbar^2$$

Very frequently the mass M used to define μ will be obvious from the context but, if there is any question, M should be defined each time H_R is used. There does not seem to be a convenient symbolism to cover all the possible uses of H_R.

Finally, there are certain other situations in which the Hartree units are modified in some relatively simple fashion. The best example is perhaps the use of $Z^{\frac{1}{2}}e$ as unit of charge instead of e in certain perturbation treatments of isoelectronic sequences. We suggest that such modified energies be called 'modified Hartrees' and denoted by H_M, or perhaps H_Z, and that the particular modification should be carefully defined in each paper in which it is used and the conversion factor to Hartrees stated. It should be noted that since mass and charge are both taken as basic units, the reduced mass and the modified charge produce independent alterations of the energy and give a modified reduced Hartree, H_{MR}.

There are difficulties of exactly the same nature with the atomic unit of length. The most usual symbol, a.u., is cumbersome and unhelpful while the symbol a_0, although it is more descriptive, is unnecessarily cumbersome since the subscript now has historical meaning only. This unit is usually called the first Bohr radius, and we should like to suggest that this be abbreviated to the 'Bohr' and denoted by b so that, in terms of the basic units :

$$b = \hbar^2/me^2$$

In exactly the same way as for the energy, the use of μ will lead to the reduced Bohr, b_R, and of a different charge to a modified Bohr, b_M.

Probably the next most useful unit for theoretical molecular calculations is the electric moment. We suggest that the appropriate unit should be :

$$eb = \hbar^2/me$$

but we do not wish now to suggest any special name or notation for it.

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Strength of Glass Fibres

An attempt has been made to eliminate the effect of surface discontinuities on the tensile strength of glass fibres by producing specimens coated with a glass which at elevated temperatures behaves as a viscous liquid whilst the core remains a brittle solid. Assuming some inter-diffusion at the boundary between the two glass compositions it was expected that the silicon oxygen random network structure of the two glasses would be continuous across the boundary and that there would be a gradual transition, on the molecular scale, from the solid to the liquid phase.

These ideal concepts are difficult to realize under experimental conditions. Besides the necessary viscosity-temperature relationships it is also required that the thermal expansion coefficients should match closely or alternatively be such that the coating is in compression when the specimen is cold. Otherwise there is a tendency for specimens to fracture spontaneously during cooling. In addition there should be no tendency towards phase separation at the boundary due to mixing of the two glass compositions.

Specimens have been produced having a core of composition by weight per cent of CaO, 23·3; Al_2O_3, 14·7; SiO_2, 62·0 and a coating of 'Pyrex' glass and tensile strength tests have been made on them at 700° C. The compositions fulfil the first two requirements outlined above, but there is an apparent tendency towards phase separation under certain conditions and this limits the value of heat treatment in promoting diffusion at the interface.

Coated fibres about 3 in. long and 0·05 in. thick have been produced by normal glass-working techniques and these were afterwards drawn down at their centres to produce a test length of fairly uniform diameter about ½ in. long. The ends of the fibres were heated to form beads which served as attachment points for stainless steel clamps by which the fibres were loaded. The samples were supported in an electric tube furnace so that the test length of the fibre was at a uniform temperature of about 700° C. They were then loaded by allowing water to flow slowly into a container attached to one end of the fibre. The time taken to fracture averaged about 10 min.

The stress at rupture for each specimen was calculated from a knowledge of the load applied and the cross sectional area of the core of the fibre. The latter was evaluated from a microscopic examination of the fracture surface of the specimen. The boundary between the two compositions could be distinguished quite easily by suitable illumination.

The experimental results shown in Fig. 1 are for fibres varying in diameter from 0·002 to 0·010 in. Over this range the effect of fibre thickness noted by previous workers[1,2] is small compared with the differences found to exist between coated and uncoated fibres. The variability of the results is very great; but the maximum tensile strength values of the coated fibres was found to be several times greater than that of uncoated specimens of the same thickness.

An attempt is being made to evolve compositions which will eliminate any tendency towards crystal growth at the interface due to mixing of the two compositions and which will enable specimens to be manufactured under more closely controlled conditions.

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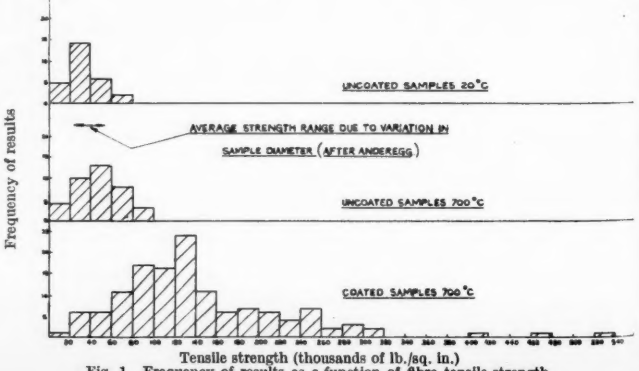
Frequency of results
UNCOATED SAMPLES 20°C
AVERAGE STRENGTH RANGE DUE TO VARIATION IN SAMPLE DIAMETER (AFTER ANDEREGG)
UNCOATED SAMPLES 700°C
COATED SAMPLES 700°C
Tensile strength (thousands of lb./sq. in.)

Fig. 1. Frequency of results as a function of fibre tensile strength. Thickness range $2 - 10 \times 10^{-3}$ in.

An Image-Splitting Microscope for Accurate Measurement

An instrument has been devised for the very precise measurement of small objects, such as thin wires, blood cells or the like. The principle involved, which is a century and a half old, is that of the heliometer. The image is split into two complete images which may be sheared across each other by operation of a suitable control. The two images of the object are set edge to edge, an adjustment which is capable of great accuracy, and the amount of shear involved is read off from a suitable scale. The shear is then reversed, crossing the two images over and setting them edge to edge once more. The difference between the two readings of shear is proportional to the diameter of the object and can be calibrated by means of a 'stage micrometer'.

In the model which has been constructed, the shear is effected in an attachment to the eyepiece. This contains two prism blocks which form an optical system similar to that of a Mach-Zehnder interferometer. The two blocks are rotated with respect to each other about a vertical axis by means of a micrometer drive, giving a shear which is strictly proportional to the micrometer reading. Fig. 1 is a photograph of a blood smear, showing a few cells which have been sheared into edge-to-edge contact,

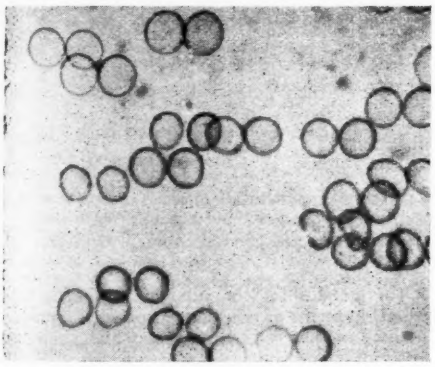

Fig. 1. Human blood cells under image-splitting microscope (×800)

and others which are not in contact or which overlap.

Using a 4 mm. objective and a 15× eyepiece, repeated settings can be made on a single cell with a standard deviation of one or two micro-inches, that is, about 0·5 per cent of the diameter of the red cells.

Objects of a periodic nature, such as wire helices, can be very rapidly checked for pitch errors by shearing the images of corresponding elements of structure in successive periods into coincidence and passing the object longitudinally under the microscope. Small pitch errors are then immediately evident to the eye and can be measured precisely when detected.

As the only modification to the microscope is in the eyepiece, no restriction is placed on the method of microscopy or the powers used.

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CHEMISTRY

Evidence for a Hydrate of Cellulose from Studies of its Surface Properties

During an investigation of the surface properties of cellulose the relationship between the contact angle of water on cellulose and the water content of the cellulose was determined. The determination of the water-cellulose-air contact angle was done with the sessile drop method on regenerated cellulose films, and with the vertical rod method using thick cellulose fibres. (The model filaments of P. H. Hermans[1]). The contact angle of water on cellulose was determined at 20° C. in a gas phase consisting of air with a relative humidity with which the cellulose films and fibres of different water content were in equilibrium. Changes in the water content of the cellulose, ranging from 0 to 25·9 per cent absorption, were obtained by keeping the cellulose at 20° C. in air of different relative humidities varying from 0 to 95 per cent.

The water-cellulose-air contact angle was found to vary as a function of the water absorption of the cellulose, or the relative humidity of the air, as shown in Fig. 1. The numbers on the curve refer to the relative humidities with which the cellulose was in equilibrium at the various water absorptions.

From this contact angle-water absorption relationship it can be seen that the contact angle is almost constant from nil to about 12 per cent water absorption, whereas there is a sharp decrease in the contact angle when the water absorption of the cellulose increases from 12 to 25·9 per cent. This shows that when the relative humidity of the air is increased from 0 to 95 per cent, the first 12 per cent water absorption has a negligible effect on the surface properties of cellulose, but a further 13·9 per cent water absorption causes the contact angle to be reduced from 24·4° to 13·4°.

The hydrophilic character of cellulose, as revealed by the water-cellulose-air contact angle, is governed by the number, the steric arrangement and the accessibility of its hydroxyl groups. Probably because of its submicroscopic crystalline structure a certain number of its hydroxyl groups are not accessible to water, and its hydrophilic character was found to be less than expected. Water absorption by cellulose, which causes its total number of hydroxyl groups to increase, ought therefore to increase its hydrophilic character and to reduce its contact angle with water. That cellulose could take up about 12 per cent water with only a negligible effect on its surface properties, indicates that this amount of water is firmly bound to

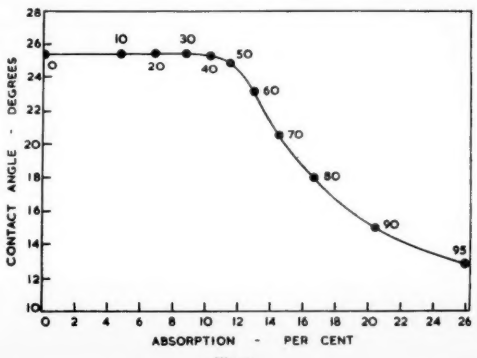

Fig. 1

cellulose by the same strong forces as in a true hydrate, and in such a way that the number of free, accessible hydroxyl groups is not markedly increased. When the number of sites in the cellulose capable of forming true hydrates is fully occupied, the additional amount of water which can be absorbed seems to be held by weaker forces and to form a loosely absorbed layer of water molecules. In this way the number of the hydroxyl groups is increased and the cellulose rendered more hydrophilic as is shown by the sharp decrease of the contact angle above 12 per cent water absorption.

The definite difference in the way water is held by regenerated cellulose below and above 12 per cent absorption is in good agreement with, and is a further support for, the conclusion which P. H. Hermans has drawn from X-ray diffraction studies on regenerated cellulose, namely, that such cellulose can take up 12·1 per cent water as a true hydrate[1].

Further details will be published elsewhere.

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BIOPHYSICS and BIOCHEMISTRY

The Optical Rotatory Power of Collagen

The changes in the optical rotatory power of collagen in solution on thermal denaturation have been extensively studied[1-3]. Cohen[1] first showed that the optical rotatory dispersion curve for undenatured collagen is normal, that is, is fitted by a single term Drude equation. This result was confirmed by Harrington[4] for three different collagens. The observation of normal dispersion, despite the fact that the collagen molecule in solution is known to have a similar three-chain helical configuration to that found from X-ray fibre diagrams, is in contrast to the observation of anomalous dispersion allied to helical configuration in the α-proteins and α-synthetic polypeptides.

The importance of the amino-acid residues proline and hydroxyproline (the pyrrolidine residues) in the configuration and optical rotatory power of proteins has been discussed by Szent-Györgyi and Cohen[5]. The relationship between optical rotatory power and the content of pyrrolidine residues has been studied in this work by measurements on the optical rotatory properties of a number of soluble collagens ('procollagens') with different contents of the pyrrolidine residues. Various values of the specific optical rotations for sodium *D* light for different undenatured and denatured collagens are given in the literature, for example, values quoted for undenatured calf skin collagen are −350°C.[4] and −415°C.[3] and for undenatured ichthyocol −330°C.[4] and −350° ±30°C.[1] No systematic study of the optical rotatory properties of different collagens has previously been reported where measurements from collagen to collagen have been made under closely similar conditions.

The range of pyrrolidine content in soluble collagens lies between 160 and 225 residues per 1,000 total amino-acid residues, and to cover this range measurements were made on collagens extracted from the swim bladders of cod and perch and the skins of cod, herring, eel and rat.

The extraction procedure used (at 4°C.) was developed by Orekhovich, Toustanovski and Plotnikova[6]; final solutions were made in a citrate-hydrochloric acid buffer at *p*H 3·7 (μ=0·075). To minimize errors in polarimetry the solutions were as concentrated as possible (0·2–0·4 per cent) and clarified by centrifugation at $10^5 g$ for 2 hr. at 4°C. Optical rotation readings were made using a Hilger standard polarimeter, a 4 dm. tube, and sodium *D* light at the temperature specified (Table 1); the temperature of measurement was controlled to ±0·2°C. Each optical rotation reading was repeated at least 20 times and the results analysed statistically; results were obtained on two independent preparations. The concentrations of the solutions were measured in terms of the nitrogen content. The total percentage nitrogen for each collagen was determined by a dry weight micro-Kjeldahl method (three determinations) and the solution nitrogen also by the micro-Kjeldahl method (four determinations).

The specific optical rotations for sodium *D* light $[\alpha]_D$, both before and after thermal denaturation are given in Table 1; the errors represent the deviations from the mean results. A value for $[\alpha]_D$ for undenatured cod skin is not given since this material, after optical clarification, was found (from its intrinsic viscosity value) to be partially denaturated.

Table 1. The specific optical rotations of undenatured and denatured collagens in solution

Extracted Collagen	$(\alpha)_D$ undenatured	$(\alpha)_D$ denatured	T_D(°C.)
Cod Skin	—	−109·7°±0·5° (40°C.)	13*
Cod Swim Bladder	−397°±4° (6°C.)	−116·0°±1·2° (40°C.)	16·5
Herring Skin	−408°±5° (6°C.)	−124·5°±1·5° (40°C.)	18·0
Eel Skin	−398°±3° (6°C.)	−125·7°±0·9° (40°C.)	26·0
Perch Swim Bladder	−400°±6° (16°C.)	−127·5°±1·1° (40°C.)	31·3
Rat Skin	−409°±8° (16°C.)	−135·0°±1·7° (50°C.)	36·5

* Taken from ref. 5.

The thermal stability of collagen in solution may be described[2] in terms of the 'denaturation temperature', T_D, defined as that temperature at which the intrinsic viscosity of the collagen has decreased to half its original value. We have shown[7] that there is a linear relationship between T_D and pyrrolidine content for collagen and the latter is represented in Table 1 by the value of the denaturation temperature.

Evidently, within experimental error, the optical rotatory power of this series of extracted collagens before denaturation is independent of the content of pyrrolidine residues. The fact that the values of $[\alpha]_D$ for the different collagens were measured at different temperatures does not affect this result since the temperature dependence of $[\alpha]_D$ for collagen over the ranges of temperature adopted is small. The constancy of the specific optical rotation indicates, within the limits of the changes in amino-acid composition of the collagens as the pyrrolidine content changes, that the optical rotation per residue in the environment of the collagen helix is independent of the nature of the residue. The optical rotatory power appears to be fundamentally a property of the helix rather than its chemical composition and is unrelated to its thermal stability.

For the thermally denatured collagens there is a systematic increase of $[\alpha]_D$ with pyrrolidine content. This variation is not unexpected since it is considered that after denaturation the polypeptide chains of the

collagen molecule are separated and each chain adopts a randomly coiled configuration[2,3]. As the content of the pyrrolidine residues increases, presumably the number of pyrrolidine—pyrrolidine links between the glycine residues (which in the latest collagen models appear at every third residue position along each polypeptide chain for stereochemical reasons) also increases, and there is an increasing tendency, at least along short lengths of the polypeptide chains, for a poly-L-proline II type configuration[8] to be maintained. In the solid state the poly-L-proline II helix is known[8] to be left-handed with an exact three-fold screw axis in the chain direction; recent polarimetric studies have shown that this configuration is largely maintained in solution. Aqueous solutions of poly-L-proline II are characterized by very high lævo-rotatory power ($[\alpha]_D^{25} \sim -500°$).

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Some Improvements in Colorimetric Determination of Elastin

A method was previously developed[1] for the direct colorimetric estimation of elastin by treating a sample of tissue with orcein. All free stain is removed and elastin hydrolyzed with elastase (pancreatin). The stain which is brought in this way into solution is then photometrically estimated.

The method is reported here with some further improvements that made it possible to determine age variations in the elastin content of human lung[2] and aorta[3].

The following reagents are used: (1) 95 per cent ethanol containing 0·1 ml. concentrated hydrochloric acid per 100 ml.; (2) orcein stock solution: orcein 1 gm., anhydrous ethanol 100 ml., concentrated hydrochloric acid, 1 ml.; (3) before use, dilute 1 ml. stock solution with 9 ml. reagent 1); (4) glycocoll-NaCl-NaOH solution (Sörensen buffer), *p*H 9·5 at 38°C.; (5) 4 per cent pancreatin solution, prepared before use with Sörensen buffer 4). Filter until thoroughly clear.

Defatting and drying the tissues. The tissues (about 1·5 gm. fresh weight) are finely minced to 2-mm. particles and washed with water to remove blood. The tissues are then extracted at 40°C. for 8 hr. with 3 changes of 100 ml. acetone and dried at 70°C. for 12 hr.

Maceration. The dry matter is finely triturated in a mortar and a sample 30–50 mgm. is macerated in a 25-ml. glass-stoppered tube with 10 ml. 0·1 *N* sodium hydroxide at 25°C. for 8 hr. with frequent mixing.

Staining and washing. The tube is centrifuged and the residue suspended twice in 10 ml. of acid ethanol (reagent 1) at 38°C. for 30 min., centrifuged and treated with 20 ml. orcein solution (reagent 3). Staining is carried out at 25°C. for 24 hr. with occasional stirring, then the mixture is centrifuged and the residue repeatedly washed with 20 ml. acid ethanol (reagent 1) at 38°C. until the free colour is completely removed. A first washing of 2 hr., a second of 12 and a third of 5 are generally sufficient. Ethanol is removed by centrifuging and washing the residue with 10 ml. of buffer (reagent 4) at 38°C. for 1 hr. The buffer is then replaced by exactly 5 ml. of fresh buffer.

Enzymatic digestion and colorimetric estimation. Digestion is carried out by adding to the tube 5 ml. of 4 per cent pancreatin solution (reagent 5) and incubating with frequent stirring at 38°C. for 1 hr.

All undigested materials are centrifuged. A slight turbidity due to the pancreatin solution which may persist has no appreciable effect. Optical density is read at 590 mμ. As a blank in the colorimetry, a control tube with 5 ml. buffer and 5 ml. pancreatin solution is treated in the same way. Colour is completely stable.

The percentage of elastin in the sample may be obtained from the following formula (previously calculated by a calibration curve with pure elastin[1]):

$$\text{Percentage elastin} = \frac{D \times 1540}{P}$$

where D is optical density and P the dry weight of the sample in mgm.

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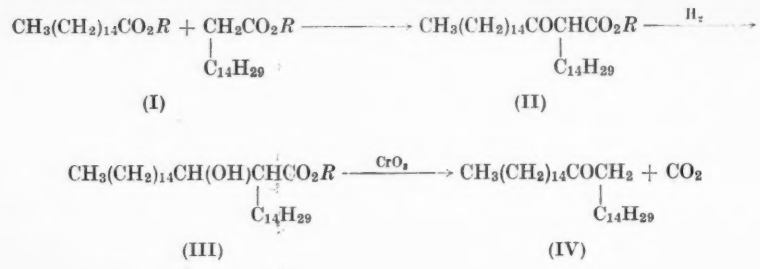
Biosynthesis of Corynomycolic Acid from Two Molecules of Palmitic Acid

Corynomycolic acid, $C_{32}H_{64}O_3$, m.p. 70°, $[\alpha]_D = +7·5°$ has been isolated from the lipids of *Corynebacterium diphtheriæ*[1] and *C. ovis*[2]. Its structure (III, $R = H$) has been established by degradation[3] and confirmed by synthesis: the condensation of two molecules of methyl palmitate (I, $R = CH_3$) in presence of NaH gives the β-ketoester (II, $R = CH_3$) which is reduced by $NaBH_4$ to a mixture of two diastereoisomers (III, $R = CH_3$)[4]; one of these has been shown to be racemic methyl corynomycolate[5].

The presence of free palmitic acid and of palmitone (IV)[6] in the lipids of *C. diphtheriæ* as well as previous work on the mycolic acids of Mycobacteriæ[7] suggested that corynomycolic acid (III, $R = H$) is formed *in vivo* by the condensation of two molecules of palmitic acid (probably as palmitoyl-coenzyme *A*) and reduction of the intermediate β-ketoester (II). Palmitone (IV) could be formed through saponification and decarboxylation of the β-ketoester (II)[8,9].

The following experiments seem to prove that corynomycolic acid is, in fact, synthesized *in vivo* from two molecules of palmitic acid.

C. diphtheriæ (strain Parke–Williams 8) was grown in 500 ml. of Loiseau–Philippe medium containing 10 μc (total activity $14·4 \pm 2·0 \times 10^5$ counts/minute) of the potassium salt of 1-^{14}C palmitic acid (specific activity $43·1 \pm 6·0 \times 10^7$ counts/min./m. mole). After 12 days at 37°, the cells were centrifuged, washed and extracted with alcohol-ether (1 : 1).



(I) (II)

(III) (IV)

Radio-activity was measured by plating infinitely thin samples on aluminium planchets and counting directly with a thin window Geiger-Müller tube. The errors made in weighing, diluting, counting and plating were taken into account and expressed as twice the standard deviation.

From 712 mgm. of alcohol-ether soluble extract (total activity approximately $32{\cdot}3 \times 10^4$ counts/min.) 160 mgm. of saturated lipids (total activity approximately 24×10^4 counts/min.) were separated by cristallization in acetone and centrifugation at $-10°$.

Corynomycolic acid (III, $R = H$) was isolated from the saturated lipids by chromatography on magnesium silicate-celite, esterification with diazomethane, distillation under reduced pressure and finally chromatography of the methyl ester on alumina; 3·4 mgm. of nearly pure methyl corynomycolate (approximately 35×10^3 counts/min./mgm.) were eluted with benzene-ether (1 : 1). This ester (III, $R = CH_3$) was recrystallized several times, first in methanol, then in acetone and an unidentified product m.p. = 65·5 – 66·5° of weak activity (approximately $70{\cdot}1 \times 10^2$ counts/min./mgm.) was separated.

The mother-liquors were chromatographed on magnesium silicate-celite, and finally two fractions were obtained which contained together 3 per cent of the initial radio-activity of the palmitic acid added to the culture medium:

(*a*) eluted in hexane-benzene (3 : 1) total activity approximately $12{\cdot}4 \times 10^3$ counts/min. m.p. 54·5–60°.

(*b*) eluted in hexane-benzene (1 : 1) : total activity approximately $34{\cdot}8 \times 10^3$ counts/min., m.p. 58–62°.

Fraction (*b*) was recrystallized in acetone and $0{\cdot}398 \pm 0{\cdot}0013$ mgm. of pure methyl corynomycolate (III, $R = CH_3$) of melting point 59–62·5° and specific activity $44{\cdot}7 \pm 4{\cdot}5 \times 10^3$ counts/min./mgm. or $22{\cdot}8 \pm 2{\cdot}3 \times 10^6$ counts/min./m.mole were isolated. The purity of the compound was further demonstrated by cochromatography on magnesium silicate-celite with authentic methyl corynomycolate ($23{\cdot}7 \pm 0{\cdot}4$ mgm.) which was added as carrier at this stage. $24{\cdot}1 \pm 0{\cdot}4$ mgm. of corynomycolic ester (specific activity $36{\cdot}6 \pm 4{\cdot}4 \times 10^4$ counts/min./m.mole) were eluted in hexane-benzene (1 : 1). Taking into account the 61-fold dilution with the carrier and the experimental errors, the specific activity of the ester is constant ($22{\cdot}3 \pm 2{\cdot}7 \times 10^6$ counts/min./m.mole). A fraction of the mother-liquors of crystallization of (*b*) (total activity $59 \pm 5{\cdot}9 \times 10^2$ counts/min.) was then added to this ester and this mixture was again chromatographed on magnesium silicate-celite. The hexane-benzene (1 : 1) eluate contained $20{\cdot}3 \pm 0{\cdot}4$ mgm. of ester (specific activity $61{\cdot}6 \pm 7{\cdot}4 \times 10^4$ counts/min./m.mole). After saponification, crystallization and purification of the mother-liquors (chromatography, addition of carrier and further crystallization) a total quantity of 20·2 mgm. of corynomycolic acid, m.p. 68·5–71·5° (specific activity $33{\cdot}0 \pm 4{\cdot}0 \times 10^4$ counts/min./m. mole) was obtained.

If corynomycolic acid (III, $R = H$) is synthesized *in vivo* from two molecules of palmitic acid (I, $R = H$), then, in our experiment with carboxyl labelled palmitic acid, only carbon atoms 1 and 3 of corynomycolic acid should be labelled and each should contain half of the radio-activity. To prove this, the labelled corynomycolic acid was oxidized and decarboxylated in a chromic acid-acetic acid mixture. The barium carbonate obtained (yield 72 per cent) had a specific activity of $16{\cdot}3 \pm 6{\cdot}4 \times 10^4$ counts/min./m.mole. The reaction mixture was extracted with ether and chromatographed on magnesium silicate-celite. The hexane and hexane-benzene (1 : 1) eluates contained palmitone (IV) which after recrystallization in hexane had a melting point of 78–80° and a specific radio-activity of $15{\cdot}3 \pm 2{\cdot}1 \times 10^4$ counts/min./m.mole. After recrystallization in hexane, the melting point (79–80·5°) and specific radio-activity ($16{\cdot}3 \pm 2{\cdot}3 \times 10^4$) remained practically unchanged. There was no depression of the melting point after mixture with authentic palmitone.

The location of radio-activity in this sample of palmitone will be studied in further experiments.

The equal distribution of radio-activity between the carboxyl group of corynomycolic acid (III, $R = H$) and the palmitone (IV) obtained by oxidation, seems already to be a conclusive proof for the biosynthesis of corynomycolic acid from two molecules of palmitic acid, thus confirming previous speculations[7–9] and excluding direct biosynthesis from smaller fragments.

A similar pathway of biosynthesis has been suggested for the high molecular weight mycolic acids of Mycobacteriæ: condensation of two molecules of normal C_{26} and two molecules of normal C_{18} acid to give C_{88}-mycolic acids[7–9].

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Combined Use of Polymyxin and Acetone to obtain Cell-free Enzymes

NEWTON[1] has shown that the bactericidal effect of the polymyxins may be explained by a disorganization of the cell-membrane, due to the combination of the antibiotic with ionized phosphate groups of phospholipid components of the membrane. These results suggest that polymyxin might be used to extract enzymes from micro-organisms. The present communication is concerned with the extraction of a nitro-reductase from *Nocardia V*.

Preliminary experiments showed that the nitro-reductase activity of *Nocardia V*. was inhibited only when concentrations of polymyxin were above 100 μgm./mgm. dry weight of cells. When cells were treated with this antibiotic at a concentration of 50 μgm./mgm. dry weight, very little enzyme activity (0–5 per cent) was released into the medium. On the other hand about 60 per cent of the 260 mμ absorbing material of the cell was liberated.

Cells were grown in a complex medium and harvested at the end of the exponential phase by centrifugation, washed twice with 0·85 per cent saline solution and resuspended in saline at about 10 mgm. dry weight per ml. To this suspension a polymyxin solution was added to give a final concentration of 50 μgm. per mgm. dry weight of cells. The mixture was allowed to stand for 30 minutes at room temperature and centrifuged for 10 min. at 1200 *g*. The supernatant was discarded and the cells washed once in distilled water. These cells were now resuspended in a small volume of distilled water and added dropwise to 20 vol. of ice-cold acetone which was stirred vigorously until coagulation occurred. The coagulated organisms were allowed to settle and some of the clear supernatant was removed by decantation. The rest of the material was filtered on a Buchner funnel with moderate suction. The precipitate was washed with 100 ml. of cold acetone followed by 100 ml. of cold ether; gentle suction was maintained for a few minutes to dry the preparation, which was transferred to a vacuum desiccator overnight. All manipulations were carried out at room temperature. Powders prepared in this way retained their original activity for several months when adequately preserved in a desiccator.

Preliminary experiments indicated that some nitro-reductase enzyme can be extracted from the acetone-powdered organisms in the absence of polymyxin treatment. When acetone powders are prepared as described above, but without previous treatment with polymyxin, not more than 25 per cent of the activity can be extracted by incubation for 1 hr. at room temperature in distilled water, 0·16*M* sodium chloride, 0·2*M* acetate buffer *p*H 4·5–6, 0·06*M* phosphate buffer *p*H 5·7–8, 0·1*M* pyrophosphate buffer *p*H 6–8, 0·2*M tris* buffer *p*H 7–9, or 0·2*M* borate buffer *p*H 9–10. Longer incubations at this temperature or at 30° do not increase the amount of enzyme extracted; incubation at 37°C. leads to partial inactivation of the enzyme. The amount of the enzyme extracted was doubled when acetone powders were ground for 5 min. in a cold mortar with 75 per cent saturated ammonium sulphate solution at *p*H 7·6, left at room temperature for 30 min., centrifuged for 20 min. in the cold at 2000 *g*, the sediment resuspended in distilled water, again centrifuged and the supernatant used for test.

Table 1 shows that pretreatment of the cells with polymyxin gives a very significant increase in the amount of enzyme extracted under some of these conditions. Variations in the final concentration of ammonium sulphate and the temperature of extraction do not increase the degree of extraction.

Table 1. EXTRACTION OF NITRO-REDUCTASE ENZYME FROM ACETONE-POWDERED CELLS

	% of initial enzyme activity	
	Untreated cells	Polymyxin treated cells
Total suspension	100	100
Distilled water extract	20–30	67
0·06*M* Phosphate buffer *p*H 7·6	25	56
75% saturated $(NH_4)_2SO_4$	52	86–91

Other surface-active agents such as '*CTAB*', 'Aerosol-*OT*', 'Tween 80', 'Tyrocidin' or 'Tyrothricin', have been tested in this connexion but they either proved to inhibit the enzyme at low concentrations (10 μgm. '*CTAB*'/mgm. or 30 μgm. 'Aerosol'/mgm.) or to be without significant effect on the release of enzyme.

I am grateful to Drs. C. J. Shepherd and B. A. Newton for advice and interesting discussions. My thanks are due to the Ramsay Memorial Fellowships Trust which supported this research with a grant.

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July 30.

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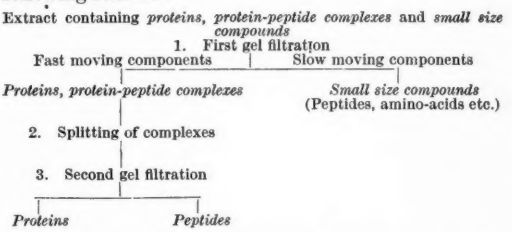
Gel Filtration as a Method for Purification of Protein-bound Peptides Exemplified by Oxytocin and Vasopressin

WE would like to call attention to a new procedure suitable for purification of simple compounds weakly associated with high molecular weight substances. The method, which was proposed elsewhere by one of us[1], is based on separation according to molecular size achievable by means of filtration through dextran gels[2]. The principle which involves three operations may be illustrated for protein-bound peptides by the following scheme:



This method is, of course, possible only when the complex being studied is stable enough to survive the first gel filtration, but capable of being easily split under other conditions. Such prerequisites may appear strongly to limit the applicability of the procedure. However, this is not necessarily the case since gel filtration can be carried out rapidly under very mild conditions[1,2]. We have chosen purification of hog vasopressin and oxytocin as a suitable example for illustration.

Acetone-dried powder (2·880 gm.) from posterior lobes of hog pituitaries ('PLP') was triturated for half an hour with 10 ml. of 0·2 *M* pyridine–0·05 *M* acetic acid buffer, *p*H 5·9. The insoluble residue was removed by centrifugation and discarded. The supernatant solution was placed on top of a column (4 × 60 cm.) of 'Sephadex *G*25' (water regain 2·5 gm.

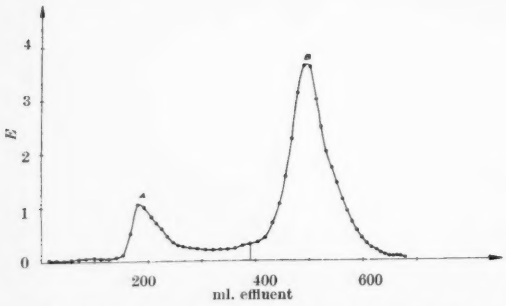


Fig. 1. Result of gel filtration of a crude extract of 2·880 gm. of posterior lobe powder. Optical density of the ninhydrin colour obtained with 0·025 ml. aliquots is plotted against effluent volume. The bulk of rat and avian pressor activity is found in over 20 per cent yield in the material of peak *A*. Approximately two-fold enrichment of activities is obtained

per gm. dry weight; obtained from Pharmacia, Uppsala, Sweden). Elution was performed with the same buffer at 180 ml. per hour. The fractions collected were analysed by the ninhydrin reaction[3]. The pooled fractions *A* and *B* (Fig. 1) were lyophilized and tested for their effect on rat and avian blood pressure[4,5]. A clear fractionation is obtained with both activities located in peak *A*.

A part of the active fraction *A* (478·2 mgm.) was treated with 5 ml. 1 *M* formic acid for 10 minutes at 70°C. in order to split the complexes. After cooling the sample was transferred to a 'Sephadex *G*25' column (2·5 × 50 cm.), prewashed with 1*M* formic acid. The gel filtration was carried out with 1*M* formic acid at a rate of 30 ml. per hour. The 5-ml. fractions collected were analysed by the Folin-Lowry reaction[6] and the ninhydrin method. The samples were pooled in three fractions, *C*, *D* and *E*, as indicated in Fig. 2 and lyophilized.

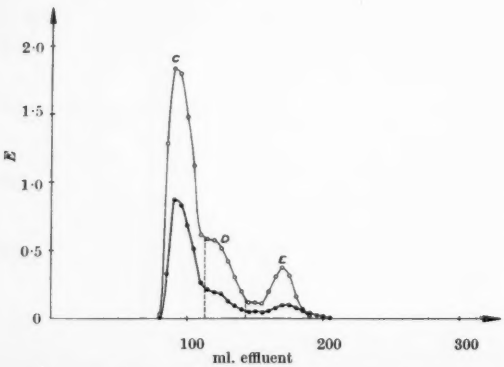


Fig. 2. Gel filtration of fraction *A* (see Fig. 1 and Table 1) after analysis with Folin-Lowry reagent (0·3-ml. aliquots; thin-lined curve) and ninhydrin (0·03-ml. aliquots). Vasopressin and oxytocin are found in good yields in peak *E*. An additional 20–25-fold purification is obtained in this second filtration

Table 1

Starting material	Initial amount	Initial activity I.U. vasopressin /mgm.	Initial activity I.U. oxytocin /mgm.	Fractions	Amount recovered	Vasopressin units per mgm.	Vasopressin units total	Oxytocin units per mgm.	Oxytocin units total
PLP	2·880 gm.	*c*.2	*c*.3	*A*	1·6061 gm.	>4<5	>6400 <8000	>4<5	>6400 <8000
				B	1·1941 gm.	*c*.0·3	*c*.350	*c*.0·3	*c*.350
A	478·2 mgm.	*c*.4·5	*c*.4·5	*C*	379·7 mgm.	<0·05	19	<0·03	30
				D	18·8 mgm.	<0·2	4	>0·05 <0·75	12
				E	16·1 mgm.	>100 <150	2000	> 75 <100	*c*.1500

This study is part of a more comprehensive investigation the results of which will later be published in full detail. We are indebted to Prof. H. Rydin and Prof. A. Tiselius for their interest and encouragement and also to N. V. Organon, Oss, Holland for generous supply of pituitary powder. The investigation has been sponsored by the Swedish Medical Research Council, the Swedish Natural Science Research Council, the Wallenberg Foundation and N. V. Organon.

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Chemical Activities of *E. coli* Adsorbed on a Resin, 'Dowex-1'

Chemical activites of bacteria are affected by a number of environmental variables, among which *p*H, substrate concentration and temperature are the most important. If around a particle of a resin there are ionic layers deep enough to cover any bacterial cells adsorbed on the resin, one would not expect that the dependence of an activity of the cells on *p*H and substrate concentration will be the same for the adsorbed cells as for the cells freely suspended in solution, as McLaren has pointed out in the case of enzymes[1].

In the previous report[2], it was noted that the optimum *p*H of succinate oxidation with cells of *E. coli* (Yamaguchi strain) adsorbed on a resin

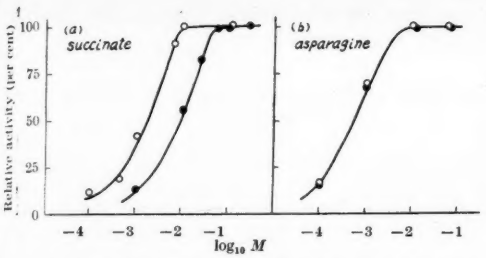

Fig. 1. Succinate and asparagine oxidation with free and adsorbed cells as a function of substrate concentration. O——O activities with free cells; ●——● activities with adsorbed cells. The maximum activities with free and adsorbed cells were taken as 100

'Dowex-1' in the chloride form was higher than that for cells freely suspended in solution. This difference could be interpreted tentatively in terms of a cationic layer outside the anionic layer at the surface of the resin and deep enough to cover adsorbed cells. Adsorbed cells in such a cationic layer would be exposed to a higher hydrogen ion concentration than cells freely suspended in solution. In such a layer the concentration of an anion would be lower than in the bulk solution. On the other hand, the concentration of a zwitterion would be almost the same in both the cationic layer and the bulk solution. This assumption was examined in the study reported here.

The organism, the resin and methods of preparation of free and adsorbed cells were the same as before[2]. The experiments were carried out manometrically at 37° C. using 0·075 *M* phosphate buffer systems. Initial velocities of oxidation with free and adsorbed cells were observed at various concentrations of sodium succinate and sodium formate as anions and asparagine as a zwitterion, at *p*H 7·0. As shown in Fig. 1 *a*, the velocity reached a maximum at a higher concentration of succinate with adsorbed cells than with free cells. In the case of formate oxidation, the situation was almost the same as in Fig. 1 *a*, that is, the velocity with adsorbed cells reached a maximum at a concentration about ten times higher than that with free cells. On the other hand, the maximum velocity of asparagine oxidation occurred at the same concentration with free and adsorbed cells (Fig. 1 *b*). These results are attributable to the effects that would be expected from the tentative cationic layer.

From the results of formate oxidation with free and adsorbed cells one may suppose that the concentration of a monovalent anion in the cationic layer would be about ten times lower than that in the bulk solution, and the concentration of a monovalent cation such as hydrogen ion would be about ten times higher in the cationic layer than in the bulk solution. If this were the case, the *p*H optimum for an activity would be expected to be higher by about one unit with adsorbed cells than with free cells. In the cases of glucose, alanine and asparagine oxidation, both the *p*H–activity curve with free and adsorbed cells for the oxidation of each substrate had a similar form, and the *p*H value for the maximum activity with adsorbed cells were higher by about one unit than that with free cells. In the case of succinate oxidation the *p*H–activity curves with free and adsorbed cells were not similar (Fig. 2). However, assuming each curve in Fig. 2 to consist of two component curves (*a* and *b*, a^1 and b^1), the difference between the two optimum *p*H's, for free and adsorbed cells, can be easily explained by the cationic layer; the *p*H values for the maxima of curves a^1 and b^1 are higher by about one unit than those of curves *a* and *b*.

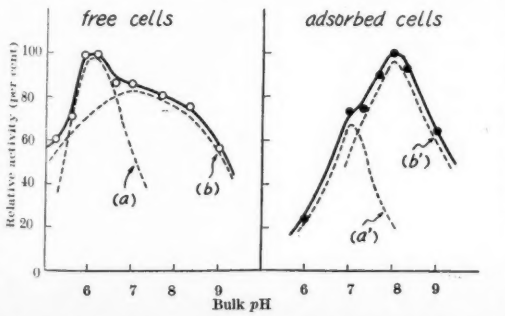

Fig. 2. Succinate oxidation with free and adsorbed cells as a function of *p*H.

Details of these experiments will be reported elsewhere.

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Paper Electrophoretic Patterns obtained by Paraffin-Oil Fixation

During experiments aimed at improving present staining techniques of paper electrophoretic patterns, it was observed that paraffin-oil is strongly adsorbed to heat-denatured proteins giving rise to water-repellent protein-dye complexes. This permitted a method to be devised by which the total area occupied by each fraction of a protein mixture, such as serum, is visualized as a sharply delimited zone against a fully white background[1].

The procedure is as follows: After conclusion of the electrophoretic experiment, the strips are air-dried and heated at 120° C. for 30 min. They are then dyed for 45 min. in a 0·1 per cent solution of bromophenol blue in 96 per cent ethanol to which 2 per cent glatial acetic acid has been added. The solution is renewed after each use. If a buffer flow indicator, such as dextran, is present, its position is marked with a pencil and the strips then submitted for 30 min. to a methanol-bath, adjusted to *p*H 4·3–4·4 with acetic acid. The paper background should remain slightly coloured. After air-drying, the strip is immersed for a few minutes in paraffin-oil and then washed lengthwise and from both sides with running tap-water at 40–50° C. for about 2 min., during which the paper is supported by a glass plate. In this way, complete removal of dye-stuff from the background is obtained. After gentle blotting, the strip is immediately placed in a hot-air oven at 120° C. and dried on crêpe-paper. Final de-oiling with trichloroethylene is recommended if the strips are to be preserved. The complete process takes about 3½ hr.

Mineral oil has been used by Freund[2] as antigen-coadjuvant to enhance and prolong antibody formation; the mode of action of the oil, however, remains obscure. Regarding the method here described, it is noteworthy that both stained and unstained native

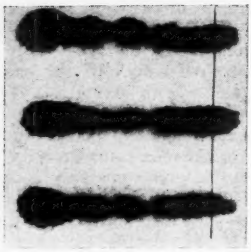

Fig. 1. Electropherogram of 3 pathological sera after oil-fixation. Electrophoretic technique as described in refs. 3 and 4. Paper, Munktell No. 20/150. Sodium acetate buffer, *p*H 5·65 and Γ/2 0·10. 3 μl. undiluted serum placed on cathodic starting-line (marked with pencil). Run : 17 hr. at 8·0 m.amp. (constant). V =1·8 V./cm.

serum proteins, as well as polypeptides, show considerably less affinity for paraffin than heat-denatured proteins. Furthermore, the slight dye diffusion into the surrounding strip (Fig. 1) has been observed in all samples treated according to this technique to occur mainly in the zone ranging from α to γ_1-globulin which contains the bulk of the lipoid components.

Fig. 1 shows three pathological sera, selected at random, and treated as described. The sharp limits seen are formed by dye concentration in the oil-water interphase during the heating to 120° C. As distinct from samples stained by other techniques, each serum shows a characteristic pattern readily appreciable at first sight. Up to five globulin fractions appear clearly distinguishable, in spite of the fact that the buffer employed is not indicated for fractionation purposes.

It will be seen that the migration end-points of the individual fractions are easily determined by visual inspection. This is of interest in mobility determinations, since results are secured without time-consuming scanning for a few hours after the end of the electrophoretic experiment.

When areas obtained by oil-fixation were measured planimetrically and the results compared with scanning data, the biggest difference was found in the albumin/γ-globulin ratio. This is because in the present method even extremely low protein concentrations seem to become visible, and therefore zones of adsorbed protein, otherwise undetected, appear especially in the γ-globulin region. On the other hand, close agreement was observed in barbital buffer of *p*H 8·6 where γ-globulin adsorption should be considerably reduced. Such concordance must, however, be viewed with caution as not only adsorption but also diffusion affects the size of the areas, while numerous and partly uncontrollable factors influence the outcome of the scanning procedure[5]. Moreover, correction values introduced to account for the different dyeing affinities of the various serum fractions may vary under pathological conditions.

In view of the shortcomings of the scanning procedure, a method based on the planimetric measurement of protein areas obtained by oil-fixation would be of interest, provided that low-concentration zones could be excluded from the areas. This might be achieved through the use of suitable carrier media, or by limiting the areas through employment of oils having less affinity for proteins. In its present form, the technique may be helpful in clinical characterizations of sera and in mobility determinations[3, 4].

The excellent technical assistance of Mr. Klaus Rasmussen is acknowledged. Thanks are due to the Fond til Lægevidenskabens Fremme for financial support of this investigation.

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June 15.

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Isomeric O-Methyl Ethers of Ethyl di-(4-hydroxycoumarinyl-3)-Acetate (the Anti-coagulant Drug Pelentane or Thromexane)

DURING [illegible] on the dependence of anticoagulant activity of the 4-hydroxycoumarine derivaties on chemical structure, it has been found, on the basis of infra-red and ultra-violet absorption spectra and the study of methylation products[1–3], that ethyl di-(4-hydroxycoumarinyl-3)-acetate (I, pelentane) and 3,3′-methylen bis-(4-hydroxycoumarine), (II, dicoumarol) show tautomeric behaviour similar to that of the derivatives of the 2-hydroxychromone (III). This equilibrium was first described by Arndt[4] in 4-hydroxycoumarine (IV). The above-mentioned equilibrium is considerably more pronounced in the *bis* derivatives than in 4-hydroxycoumarine itself.

My co-workers and I have already suggested[1,2] that the chromone component of this equilibrium is probably the cause of anticoagulant activity. To confirm this hypothesis chromone derivatives with anticoagulant effect were prepared[5–7] and from the mixture after methylation of pelentane with diazomethan three isomeric dimethyl ethers with the structures V, VI, VII were isolated. These dimethyl ethers were without biological effect, which is in agreement with the finding of Link and co-workers[7] that the chief prerequisite of activity in this group is the presence of enolic hydroxyl on the pyrane ring. In this connexion I have studied the occurence of the coumarine chromone tautomerism in mono-O-methyl ethers of pelentane and have prepared (by methylation of the silver salt of pelentane with methyl iodide) a new O-methyl ether (VIII), ethyl-(4-hydroxycoumarinyl-3)-(2-methoxychromonyl-3)-acetate, the structure of which was established by elementary analysis, determination of methoxy groups, infra-red and ultra-violet absorption spectra (Figs. 1, 2) and by comparison with O-alkyl ether of a coumarine type that was already known[8], and to which the structure IX is ascribed. The compound IX was spectroscopically characterized by an absorption band at 1,690 cm.$^{-1}$ belonging to the lactone of alpha beta unsaturated acid, whereas compound VIII has a band at 1,630 cm.$^{-1}$ belonging to the doubly conjugated CO group in position 4. The ultra-violet absorption spectra of compound IX is similar to that of 4-methoxycoumarine and the spectrum of compound VIII is similar to that of the mixed O,O′-dimethyl ether of pelentane (V).

With respect to the biological activity, a tentative comparison of the prothrombine times of both isomeric mono-O-methyl ethers with pelentane was carried out in rabbits. It was found that compound IX

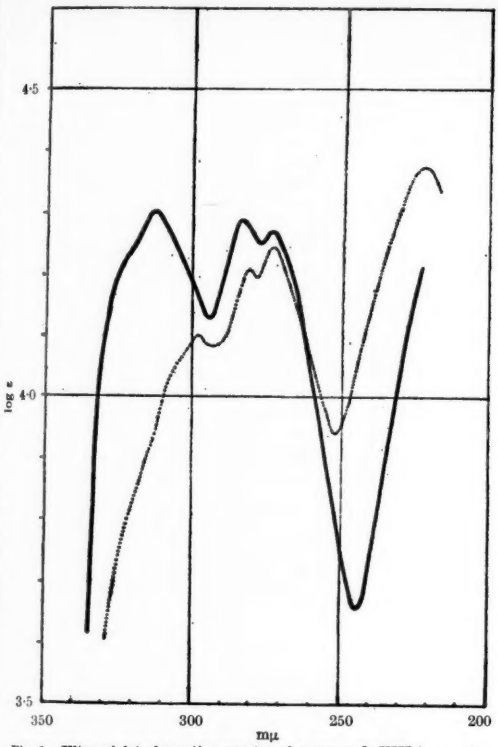


Fig. 1 Ultra-violet absorption spectra of compounds VIII (.....) and IX (———). The solvent is cyclohexane, cuvette 0·5 cm., concentration $4{\cdot}73 \times 10^{-4} M$. Ultra-violet spectrophotometer (Zeiss)

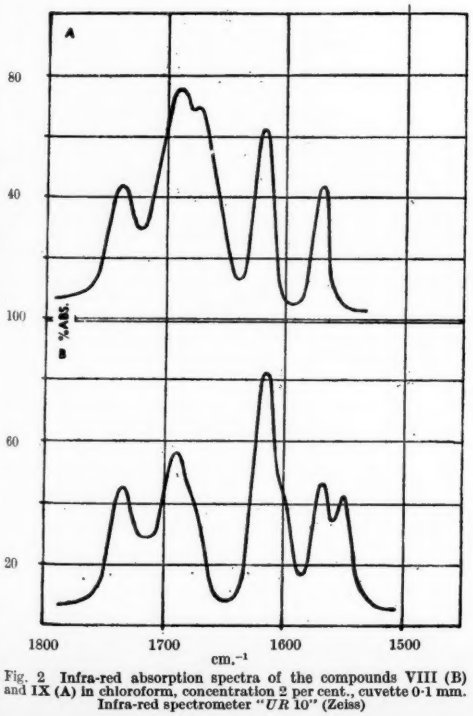
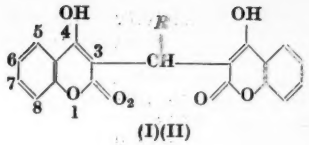


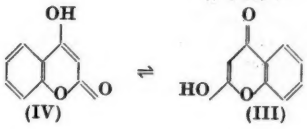
Fig. 2 Infra-red absorption spectra of the compounds VIII (B) and IX (A) in chloroform, concentration 2 per cent., cuvette 0·1 mm. Infra-red spectrometer "*UR* 10" (Zeiss)

II = dicoumarol
R = H

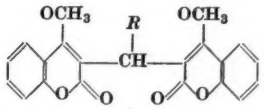
I = pelentane
R = COO C_2H_5



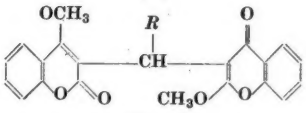
(I)(II)



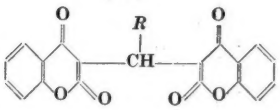
(IV) (III)



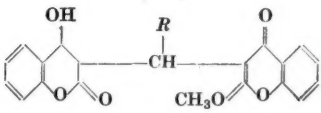
(V)



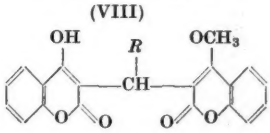
(VI)



(VII)



(VIII)



(IX)

shows considerably lower activity than pelentane, similar to that of the analogous mono-O-methyl ether derived from dicoumarol tested by Link and co-workers[7], whereas compound VIII has about 60 per cent of the activity of pelentane. From the results obtained it appears, in agreement with the above hypothesis, that the chromone isomer VIII has a stronger anticoagulant effect than the coumarine isomer IX.

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X-Ray Sensitivity and Target Volume of Enzyme Induction

The enzyme activity of lysine decarboxylase after induction with L-lysine in non-growing *Bacterium cadaveris* (NCTC 6578) increases nearly linearly with time[1] until the 'final activity' level is reached. The 'final activity' and the 'induction rate'—the slope within the linear range of the activity curve in Fig. 1—are characteristic for this strain and enzyme.

This investigation shows the inhibition on the induction process by X-rays. After X-irradiation the induction rate decreases by the same amount as the final activity, as can be seen in Fig. 1. The dose effect curves are plotted in Fig. 2. Both curves seem to be exponential. The inactivation dose is 7×10^4 *r*. Since the inactivation dose of the enzyme molecule itself is $1{\cdot}4 \times 10^7$ *r*.—which is 200 times larger—the investigated effect on the induction is different from the X-ray effect on the already formed enzyme molecule within the cell. According to Pollard *et al.*[2] an inactivation dose of 7×10^4 *r*. corresponds to a molecular weight of the radiosensitive unit of 20 million. The identical radiosensitivity of the 'induction rate'—which is essentially a velocity of the protein synthesis—and the 'final activity'—which is the amount of the end-product of the synthesis—suggest a possible mechanism of the induction. The mentioned identity of the dose effect curves could be explained by the assumption, that in each cell, one or several synthetic centres exist, and each centre will produce only a certain number of enzyme molecules. The single unit would be destroyed by a single hit.

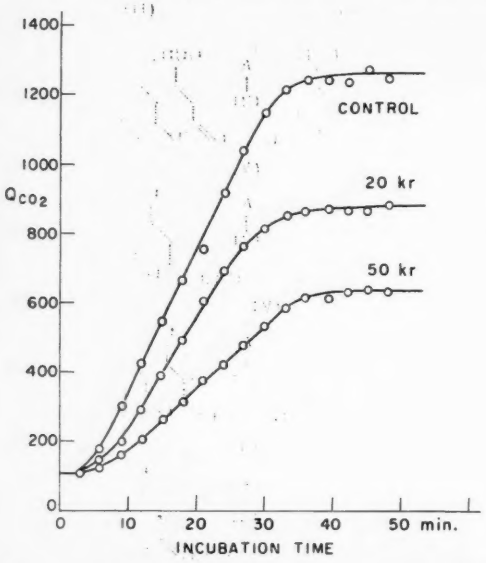

Fig. 1. Induction of lysine decarboxylase in *B. cadaveris*. The enzyme activity was measured manometrically at 30°C. in 5 vol. carbon dioxide + 95 vol. nitrogen (per cent), 3 ml. *M*/20 phthalate buffer *p*H 5·6 with 30 mgm. glucose, 1·5 mgm. Difco yeast extract and about 5 mgm. wet bacteria. Induction by addition of 200 μmol. D-L-lysine at time O. Activity unit Q_{CO_2} in mm.[3] carbon dioxide developed by decarboxylation from lysine by 1 mgm. bacteria (dry weight) in 1 hr. X-irradiation with a beryllium window tube, 45 kV., 25 m.amp., additional 0·4 mm. Al filter; dose rate at 3 cm. focus distance 2×10^4 r./min.

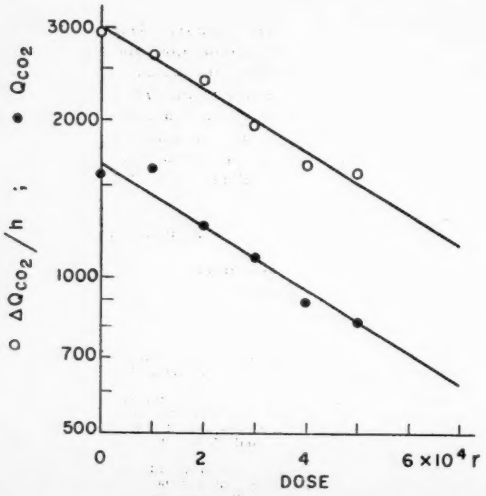

Fig. 2. Dose effect curve for the 'induction rate' (○) and the 'final activity' (●)

This hypothesis agrees with the recent findings of Novick[4] for the induction of β-galactosidase in *Escherichia coli*. The target volume corresponding to a molecular weight of 20 million, and the nucleic acid inactivation spectrum for the induction[4] leads to the conclusion that a complex nucleic acid structure is involved in enzyme induction. Approximately the same target volume has been found by Pollard[5] for the induction of β-galactosidase in *Escherichia coli*.

I wish to thank Prof. Dr. B. Rajewsky for his interest and continued encouragement.

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HÆMATOLOGY

Growth-Controlling Action of Human Serum in Cell Culture

It is reasonable to think that there are stimuli and opportunities in every human life which result in a cancerous transformation of some cells. The main factor in the progress of these cells to the clinical tumour stage might be a deficiency in the growth-controlling defensive mechanism. To demonstrate the possible existence of such factors we have studied since 1955 the effect of human serum on different cells in cell culture[1].

HeLa cells, a continuous amniotic cell line (*CAL*) and primary amnion cell cultures were used. Briefly, the technique was as follows: HeLa and *CAL* cells were cultivated in Roux bottles containing 30 per cent active human serum in Hanks solution; cell inocula were prepared by trypsinization and were cultivated in different 30 per cent human sera in Hanks solution in test-tubes. The following details are of importance for reproducible results: the inocula must be prepared from young, 1–2 day-old cultures, in excellent condition. The *p*H of the system must be controlled in all phases of the experiment (it may not exceed 7·7). The technique of cultivation of amniotic cells has been reported elsewhere[2].

Using this technique, we observed that there are great differences between fresh human sera: the mode of

growth varies; so does the cell count (multiplication rate) and size of nuclei[3]. In some human sera the cells grow in dense clumps and not in the usual loose migratory structure: in such cases the nuclei are smaller and the nuclear counts (citric acid method) usually fall after 2–4 days. Repeated bleedings during 3 years from the same individuals revealed that the mode of growth in the same dilutions of fresh serum from the same individual is stable.

The factor(s) in serum causing this clumping phenomenon are labile; they can be destroyed or their effect inhibited by, for example: (1) Heating the serum for 30 minutes at a temperature greater than 52° C. (2) Storage of the serum at + 4° C. in a refrigerator for some days. (3) Absorption of the serum with cells or zymosan. When the serum is stored in the frozen state, the factor(s) seem to be stable for at least two weeks.

A given amount of serum can change the mode of growth of a limited number of cells only, and only for a limited time. If, however, the serum dilution used as culture medium is changed repeatedly the typical mode of growth persists. Table 1 gives one typical

Table 1

	Serum *Sx*				Serum *Br*			
	Fresh		Heat Inactivated		Fresh		Heat Inactivated	
Cell counts after two days growth and one change of culture medium	86	104*	57	71	113	118	59	56
	56	79	49	60	69	63	61	60
Arithmetic mean	81		60		88		59	
Cell counts after nine days growth during which the serum dilution was changed 8 times, the cells were trypsinized 4 times, and half the trypsinized cells were used as inoculum for the same number of tubes	0	0	18	17	10	17	8	6
	0	0	9	7	5	14	7	6
	0	1	14	15	6	7	13	7
	0	0	8	11	8	11	8	8
	1	0	11	11	7	15	11	13
	1	0	4	6	4	6	4	6
Arithmetic mean	< 1		11		9		8	

The original inoculum was about 50,000 HeLa cells/ml.
* The pairs of figures represent duplicate counts from one culture tube in thousands per ml.

experiment where the culture medium was changed 8 times, the cells trypsinized 4 times, and where the nuclear counts were also followed.

There were no differences in the nuclear counts between the sera used in this case after two days' growth, but the clumping of cells in serum *Sx* was clear; in serum *Br* it was not observed. After nine days growth and repeated changes of the culture medium and trypsinization of the cells, there were only a few cells left in the test-tubes where fresh serum *Sx* was used.

These observations show that in fresh human serum there are various amounts of factor(s) which prevent cells in cell culture from growing in a loose migratory structure, which cause clumping and reduce the viability of the cells.

A question that must be studied further is the connexion between these observations made with fresh human serum and those recent observations where use is made of heterologous serum or cells[3]. It is of interest that the relatively rare type of serum with a strong clumping action was found in 5 out of 7 members of one family group. It is possible that the frequency of these sera is low among cancer patients. The observation that serum from cancer patients has a good growth-promoting effect[4] is interesting, as is the fact that greater and more prolonged growth of implanted cells occurred in cancer patients than in normal persons[5].

Investigations designed to elucidate the mechanism of the clumping and the reduction of the viability of the cells are in progress.

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Modifications in Immunological Behaviour, Electrophoretic Mobility and Colourability of Substituted Human Serum Proteins

THE azoproteins of Landsteiner were used at the beginning of immunochemical work using modified proteins to study antigen-antibody reactions. These proteins are usually controlled by the well-known quantitative precipitin reaction of Heidelberger.

Recent methods such as immunoelectrophoretic analysis and agar diffusion might be useful as screening tests for the reactivity of the substituted proteins.

We have studied the behaviour of modified human albumins and γ-globulins by zone electrophoresis on paper, starch gel and agar, by immunoelectrophoresis, and by diffusion in agar.

The substituted proteins used in the study were:

(*a*) *Derivatives of human serum albumin*: deaminated, acetylated, carboxymethylated, ninhydrinated, tosylated, phosphorylated, formolated, oxidized with performic acid, nitrated, methylated, and denatured by heating, urea and guanidine.

(*b*) *Derivatives of human γ-globulins*: deaminated, acetylated, carboxymethylated, ninhydrinated, tosylated, phosphorylated and denatured by heating and guanidine.

We found that blocking of the amino-groups, alone or combined with the introduction of new carboxylic groups, resulted in an increase of electrophoretic mobility (at *p*H 8·6) and in a decrease of colourability; blocking of the free carboxyls of albumin resulted in a decrease in the electrophoretic mobility and an increase in affinity for the usual acid dyes. These criteria might be useful in the appreciation of the results of the chemical substituting operations.

The immunological reactivity against horse or rabbit anti-human sera is not impaired by the blocking of the free amino groups. The carboxymethylated proteins gave apparently normal reactions with several antisera but failed to give a reaction with one horse and two rabbit antisera having a weaker antibody titre.

Several authors report[1,2] that carboxymethylated albumin fails to react with the specific antisera to produce a precipitate. In further studies of this problem we neutralized the antibodies with carboxymethylated protein (80 mgm. in 1 ml. of rabbit antiserum). The resulting neutralized antiserum gave no further reaction with carboxymethylated protein but precipitated the native protein. On the other hand the treatment of antiserum with native albumin (40 mgm./ml.) suppressed the reaction against the modified protein.

Esterification of the carboxylic groups of the antigen seems to have more effect on the antigen-antibody reaction. Immunological response decreases with progressive methylation. A similar result has been reported by Maurer[3]. Oxidation

of the S-S grouping in human albumin also abolishes immunological activity. This reaction (oxidation by performic acid) will be studied further. The complete results will be published elsewhere[4].

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ANIMAL PHYSIOLOGY

Relationship between pH and Volatile Fatty Acid and Diversion of Saliva from the Actively Fermenting Rumen of Sheep

The importance of a copious flow of saliva in ruminant digestion, especially with regard to the bicarbonate-phosphate buffer system[1,2] and the sodium economy of the sheep[3] is well known. Recently the total mixed saliva section rate for previously starved sheep consuming various roughage rations has been measured by the use of œsophageal fistulæ and found to vary between 2 and 12 ml./min., with a mean value of 6·1 ml./min. (17 observations) (McManus, unpublished data).

The successful use of an œsophageal fistula technique for obtaining samples of the ingesta selected by freely grazing sheep, results, over the collection period, in a substantial portion of the saliva secreted being diverted from the rumen and might be expected to interfere with the rumen environment, in particular the *p*H and volatile fatty acid pattern during digestion.

A series of four independent experiments using rumen cannulated sheep was conducted. The animals were starved for 24 hr. and then offered a measured weight of a known low lactic acid producing ration (55 per cent wheat grain, 25 per cent wheaten chaff, 20 per cent lucerne chaff). The animals had been trained to consume the ration as quickly as possible and had been on this diet for periods greater than 20 weeks prior to sampling. The sheep were denied access to water during the collection periods.

Each experiment consisted of two sampling periods on each sheep :

(*a*) During the first period, the animals, after consumption of the ration were anæsthetized ('Kemithal', lateral recumbancy) for periods up to 5 hr. and the flow of saliva diverted, collected and measured.

(*b*) During the second period, the animals consumed approximately the same amount of a similar ration, but were left in a conscious state in their pen and the saliva allowed to pass normally to the rumen.

With anæsthetized animals the saliva does not flow to the rumen because the normal swallowing mechanism is suppressed and because of the animals' position. Conscious sheep in which saliva was diverted from the rumen by means of an œsophageal fistula gave identical results.

Collection of saliva from the anæsthetized sheep and addition to the rumen fluid by way of the rumen cannula would result in incomplete mixing.

Rumen liquor samples were taken at successive short time intervals commencing prior to feeding, the *p*H determined, and analyses made for total volatile fatty acid content.

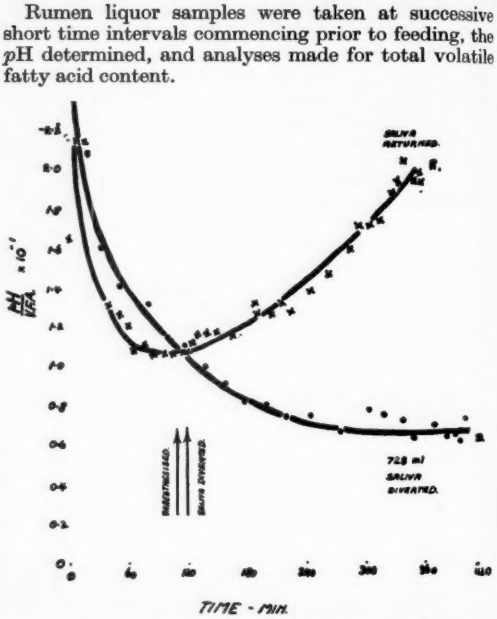

Fig. 1. The depressed *p*H/volatile fatty acid ratio obtaining over time after diversion of alkaline saliva from the rumen, in contrast to the normal behaviour of this function when saliva is not diverted from the fermenting rumen.

Data have been obtained from four such experiments on two normal animals at two levels of feed intake, and on one occasion using an animal with an œsophageal fistula.

Data from a typical experiment where 728 ml. of saliva were diverted are presented in Figs. 1 and 2.

When the *p*H/volatile fatty acid ratios were

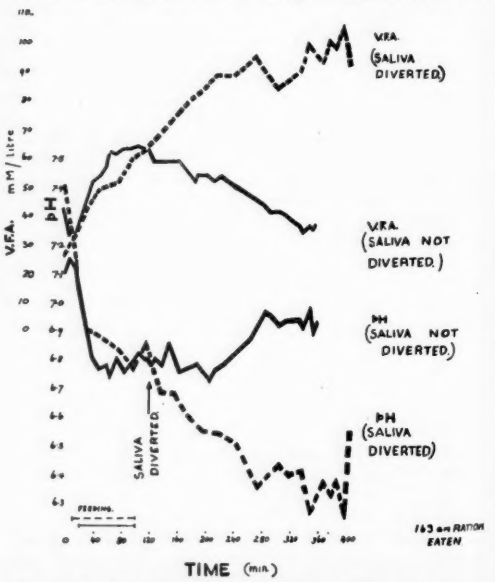

Fig. 2. The intact lines illustrate the normal *p*H/volatile fatty acid pattern described after consumption of food. The broken lines portray the abnormal elevation of volatile fatty acid and depression of *p*H upon diversion of 728 ml. of saliva. Note that the inverse relationship between *p*H and volatile fatty acid obtains in both cases.

plotted against time, comparison of the two curves indicated that a profound change in rumen *p*H and volatile fatty acid occurred some 40–60 min. after effective diversion of the saliva (Fig. 1) with the *p*H values of the rumen liquor falling normally but remaining at a low level after 4–5 hr. (Fig. 2). The volatile fatty acid values rose and remained high. By comparison, the rumen fluid in the 'saliva not diverted' treatment followed the usual pattern by returning to normal levels after attaining a trough and a peak value for *p*H and volatile fatty acid respectively.

These data indicate that the change in *p*H-volatile fatty acid relationship can be ascribed to the diversion of saliva, and would seem to constitute the first recorded direct *in situ* evidence for such a saliva effect. While not unexpected, this observation is of considerable interest since it is probable that the diversion of saliva caused either an alteration in the phosphate/bicarbonate buffer-levels obtaining in the rumen fluid; a direct or mediated depressive effect on absorption of volatile fatty acid by the rumen epithelium; or some interaction of these. The effect is not thought to be the result of a concentration effect *per se* due to the reduction of volume of fluid in the rumen, since an approximate allowance was made in the volume of rumen liquor removed for analysis.

Further studies are being made on this effect.

I wish to thank Dr. D. F. Stewart, officer-in-charge, C.S.I.R.O. McMaster Animal Health Laboratory, Sydney, for the use of animal house facilities. It is a pleasure to record the skilled technical assistance of Mr. F. J. Hamilton, senior technical officer, also of the Laboratory.

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8-Hydroxyquinaldic Acid: the Red Fluorescent Substance in the Urine of Pyridoxine-Deficient Rats

During paper chromatographic examination of urine from pyridoxine-deficient rats, an orange-red fluorescent spot was observed when the chromatograms were examined with ultra-violet light having a predominant wave-length of 2540 A. The physical and chemical properties of the compound were found to be different from xanthurenic acid, its conjugates[1,2] or any of the well-known metabolites of tryptophan which were excreted in increased amounts during pyridoxine deficiency[3]. However, the fluorescence of the compound was very similar to 8-hydroxyquinaldic acid, which was found in urine of normal rabbits[4] or in rat urine after the administration of xanthurenic acid[5]. Hence, an attempt was made to isolate and identify the compound.

The urine of rats maintained on a pyridoxine deficient diet was collected. The purified diet described by Brown, Miller and Miller[6] was used except that vitamin-low casein was used at a level of 20 per cent and *p*-aminobenzoic acid and pyridoxine were omitted from the diet. In addition, this diet contained 1 per cent L-tryptophan.

For the isolation of 8-hydroxyquinaldic acid, two 24-hr. urine collections from 12 rats were pooled. The pooled urine was diluted with water, filtered and the *p*H was adjusted to 7·0 by the addition of alkali. The urine was then passed through a column of 'Nuchar *C*' (40–200 mesh) 3·0 cm. in diameter and 23·0 cm. long. The charcoal column was then washed with 50 ml. water followed by 1,500 ml. of 0·01 *M* ethylenediaminetetraacetic acid (*p*H 7·0) as described by Pontis, Cabib and Leloir[7]. Most of the xanthurenic acid and other tryptophan metabolites were eluted with 4·5 l. of 50 per cent (v/v) of ethanol. Then 8-hydroxyquinaldic acid along with a small amount of xanthurenic acid was eluted with 1·5 l. of 5 per cent pyridine (v/v). The pyridine effluent was evaporated under reduced pressure, and 8-hydroxyquinaldic acid was isolated and purified by a previously described method[4]. The melting point, ultra-violet spectra, R_F values and colour reactions of the isolated material were found to be identical with the authentic compound[5].

For the determination of 8-hydroxyquinaldic acid in rat urine, a 24 hour urine sample was diluted with water and 1 *N* hydrochloric acid so that the final concentration of acid was 0·2 *N*. The acidified urine was passed through a column of 'Dowex 50' $(H^+)^5$ 1·6 cm. in diameter and 8·0 cm. long. To determine the recovery of 8-hydroxyquinaldic acid, a duplicate urine sample containing 610 μgm. of 8-hydroxyquinaldic acid was used. The columns were then washed with 100 ml. of 0·2 *N* hydrochloric acid, 200 ml. of 0·5 *N* hydrochloric acid and 50 ml. water. The columns were eluted with 2 l. of water and the effluent was passed directly into a column of 'Dowex 2' $CH_3COO^-{}^4$ 1·0 cm. in diameter and 3·0 cm. long. The 'Dowex 2' column was then washed with 250 ml. of 3 *N* acetic acid and the 8-hydroxyquinaldic acid was eluted with 250 ml. of 6 *N* acetic acid. Under these conditions, 8-hydroxyquinaldic acid and some kynurenic acid were eluted and xanthurenic acid remained on the column. 8-Hydroxyquinaldic acid was quantitatively determined by a colorimetric procedure as described below. To a 5 ml. aliquot of the above 6 *N* acetic acid effluent, 3·5 ml. of 9 *N* sodium hydroxide was added with cooling and the contents were mixed thoroughly. This was followed by the addition of 1·5 ml. of 1·5 *M* phosphate buffer (*p*H 7·3). Four drops of 0·25 per cent solution of 2,6-dichloroquinonechloroimide reagent[8] in alcohol were added to this solution. A bluish-violet colour appeared within a few minutes. The optical density at 600 mμ was measured after 15 minutes in a Beckman model *DU* spectrophotometer by using a 0·5 *M* phosphate buffer solution (*p*H 7·3) containing a few drops of the above reagent as a blank. At a *p*H below 6 very little reaction occurred, while above *p*H 9 the colour was rapidly destroyed. A standard curve was run at the same time and from the standard curve the recovery and the amount of 8-hydroxyquinaldic acid in the 24-hr. urine samples were calculated. The recovery of added 8-hydroxyquinaldic acid from the urine averaged 90 per cent (range 84–95). By this method 8-hydroxyquinaldic acid could not be detected in normal rat urine or after administration of tryptophan whereas pyridoxine-deficient rats on a tryptophan-supplemented diet excreted 1·02–1·50 μmoles of 8-hydroxyquinaldic acid daily (Table 1). 8-Hydro-

Table 1. Excretion of kynurenic acid, xanthurenic acid and 8-hydroxyquinaldic acid in the urine of rats maintained on pyridoxine-deficient diets containing 1 per cent L-tryptophan

Exp. No.	μmoles excreted in urine per rat per day		
	Kynurenic acid	Xanthurenic acid	8-hydroxyquinaldic acid
1	8·42	23·40	1·02
2	12·94	29·02	1·45
3	17·10	41·03	1·50

xyquinaldic acid was readily detected by paper chromatography in basal urine of pyridoxine-deficient rats without tryptophan supplements, but the amount was too small for satisfactory quantitative analysis. The kynurenic acid and xanthurenic acid contents of the urine samples were also determined by a previously described method[9] (Table 1).

The acetic acid effluent used for the determination of 8-hydroxyquinaldic acid also contained a small amount of a conjugate of this metabolite. It was purified by paper chromatography from the solvent systems described earlier[4]. The ultra-violet spectrums, R_F values and colour reaction (blue with 2,6-dichloroquinonechloroimide reagent) were identical with 8-hydroxyquinaldylglycine. On hydrolysis with hydrochloric acid, the conjugate yielded 8-hydroxyquinaldic acid, which was identified by paper chromatography. Glycine was identified on paper chromatograms by reaction with ninhydrin and ortho-phthalaldehyde reagent[10].

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Resting and Action Potentials of Reversed Polarity in Frog Nerve Cells

The present communication deals with the resting and action potentials of the nerve cell body of the frog dorsal root ganglion immersed in barium-rich media. Dorsal root ganglia were removed from the ventral side of the vertebræ of a *Rana pipiens* together with nerves 5–10 mm. long on both sides. The preparation was immersed in a sodium free solution containing 80 m*M* barium chloride, 2 m*M* potassium bicarbonate and 1·5 m*M* calcium chloride. On penetration of the nerve cells with a hyperfine glass-pipette electrode filled with 3 *M* potassium chloride, it was sometimes found that the potential of the microelectrode suddenly rose by 20–40 mV. without being accompanied by any appreciable rise in the electrode resistance. There were also many cells which showed a resting potential (40–70 mV.) of the ordinary (that is, negative) sign.

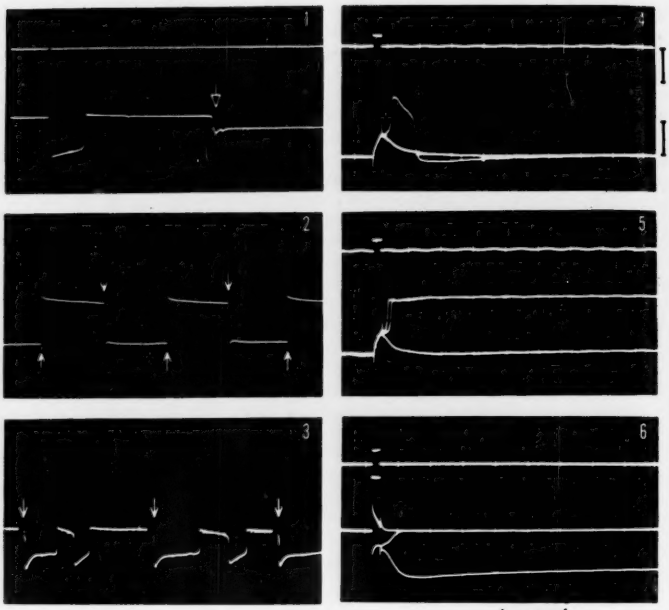

Fig. 1. Electric responses recorded from individual nerve cells in frog dorsal root ganglia immersed in barium (sodium-free) Ringer solution. The upper trace in records 1, 4, 5 and 6 represents the time course of stimuli applied to the cell; the bar on the right represents 10^{-8} A. The lower trace shows the intracellular potential (positive-upward) recorded with a microelectrode; the calibration 50 mV., applies to all the records. The large arrow in record 1 indicates the moment at which the microelectrode was withdrawn from the cell. The upwardly directed small arrows indicate delivery of cathodal (outward) current pulses and those facing downwards anodal pulses. Records 4–6 were taken from one cell; a short period of repetitive stimulation intervened between records 4 and 5 and a period of about 5 min. between records 5 and 6. On each record, three sweeps (at 2-sec. intervals) were superposed. The horizontal bar at the bottom represents 400 m.sec. for record 1, 2 sec. for record 2, 1 sec. for record 3 and 40 m.sec. for records 4–6. Temperature 22 °C.

When tested with stimulating current pulses delivered through the recording microelectrode, these nerve cells with a reversed resting potential, as well as those with a normal resting potential, were found to be 'excitable'. When the impaled cell had an ordinary (negative) resting potential, a pulse of outwardly directed current evoked a depolarizing response of 60–120 mV. in amplitude. When the impaled cell happened to show a positive resting potential (Fig. 1, record 1), only pulses of inwardly directed current through the cell membrane could evoke all-or-none responses and the polarity of the evoked potential variation was opposite to that of the normal potential. The duration of the action potential of the reversed polarity was variable; in some cases it was shorter than 10 m.sec.; but in many other cases it was longer than 100 m.sec. The peak amplitude of the response was usually 60 and 100 mV.

It is important to note that a nerve cell with a negative resting potential could be converted in many cases into a cell with a reversed resting potential simply by repetitive stimulation. As in the case of toad nerve fibres in normal Ringer[1], repetitive stimulation of the ganglion cell in the barium-rich solution prolonged the (depolarizing) action potential enormously. When a cell was stimulated at a frequency of about 10 per sec. for a period of 1–5 sec., a stage was often reached in which the plateau lasted indefinitely (Fig. 1, records 4 and 5). In this

stage, a quick withdrawal of the recording microelectrode resulted in a fall in the observed potential, indicating that the stationary (or resting) potential of the cell was now reversed. These cells were usually capable of responding to pulses of inwardly directed current with all-or-none action potentials of the reversed polarity (Fig. 1, record 6). In some of these cells, the potential could be switched back and forth between the two stable levels by delivering anodal and cathodal pulses alternately (Fig. 1, record 2).

The experimental results described in this report are regarded as additional evidence in support of the view that action potentials are produced by transition of the state of the membrane between two physicochemically stable configurations[2].

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Oestrogens in Pasture

As early as 1926 Dohrn *et al.*[1] discovered the œstrogenic activity of plant material. It was only in 1944 that Bennets[2] described disorders in sheep originating from œstrogens in pasture with a high content of subterranean clover (var. Dwalganup). Bartlett *et al.*[3] suggested that in Britain œstrogens in pasture might raise milk yield and non-fatty solids in the milk of cows.

It is, however, difficult to demonstrate œstrogens in grass by the current method, consisting of extraction of the grass and biological testing of the extracts. With this method we obtained poor results in pasture not containing subterranean clover. Prof. E. Brouwer suggested therefore that the grass should be tested as such with the aid of castrated female rats. In four such experiments we obtained good results.

Every experiment was started with three groups of ten animals three weeks old. Group I received the basal ration only, group II the same ration plus fresh grass, and group III the basal ration plus œstrone. The experiments were performed in July 1958, November 1958, April 1959 and May 1959.

The average uterus weights (mgm./gm. body weight) in groups I and II were as in Table 1.

Table 1

Experiment No.	Group I (control)	Group II (grass)	Difference
1 (July 1958)	0·098	0·174	0·076
2 (November 1958)	0·106	0·170	0·064
3 (April 1959)	0·128	0·156	0·028
4 (May 1959)	0·036	0·124	0·088

In all four experiments the difference was highly significant by Wilcoxon's test, even in the autumn grass, P being 0·001 in experiments 1, 2 and 4 and 0·004 in experiment 3.

We therefore recommend the above test for studying the œstrogenic activity of feeds more closely.

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PLANT PHYSIOLOGY

Effects of Gibberellic Acid on Juvenility in *Marsilea* and certain other Plants

In addition to the more familiar effects of gibberellic acid, it has recently been found, by several authors, that this substance can affect the duration of the juvenile stages of certain plants. Thus, Robbins[1], discovered that when an arborescent form if ivy (*Hedera canariensis variegata* var. *arborescens*) was supplied with gibberellic acid, branches with completely juvenile characteristics were produced by some of the treated plants. Robbins believed that gibberellic acid is probably comparable with the 'Jugendsubstanz' postulated by Frank and Renner[2] in explanation of their own observations, and earlier work by Doorenbos[3] on the rejuvenation of adult ivy shoots. Scurfield and Moore[4], however, found that seedlings of *Eucalyptus* species, when treated with gibberellic acid, developed the adult leaf type earlier than the control plants, although Njoku[5], using seedlings of *Ipomoea caerulea*, obtained a prolongation of the juvenile phase.

In view of these varied findings, it seemed desirable to study the effects of gibberellic acid on sporelings of the water fern *Marsilea*, a plant in which the transition from simple spatulate, followed by bifid, juvenile leaves to the quadrifid adult leaf provides a particularly clear indication of the course of heteroblastic development. In the experiments outlined here, conditions of aseptic culture were adopted because of the fine control they permit of the nutritional status of the plants. Aseptic embryos of *Marsilea drummondii* were obtained by the technique used in previous studies[6] and transferred to the various culture media. The latter consisted of a basal Knop's medium, with addition of gibberellic acid to give final concentrations of 0, 1·0, 10·0 or 100 mgm./l. A.R. glucose was supplied as an organic carbon source at concentrations of 2 or 4 per cent. The cultures were kept at a constant temperature, 22–24°C., and illuminated by fluorescent tubes giving a light intensity of approximately 150 foot-candles at the level of the cultures. Weekly records were made of leaf number, leaf segmentation, and any other morphological differences observed. Some of the results obtained are summarized in Table 1.

As in the work of other authors, moderate concentrations of gibberellic acid (1·0 and 10·0 mgm./l.) led to some increase in the growth rate of the plants, as shown by the overall size of leaves and rhizome, and the length and degree of branching of the roots; a slight decrease in the rate of leaf production was perhaps related to the greater size of the young leaves and roots of the apical region. At the 100 mgm./l. concentration of gibberellic acid, the roots were markedly stunted and the shoot system somewhat shorter than in the controls.

In the present context, the changes in leaf segmentation represent the most interesting response to the gibberellic acid. It is evident from Table 1

Table 1. Effects of gibberellic acid and of glucose concentration on leaf segmentation and leaf production in *Marsilea drummondii*

Gibberellic acid concentration in mgm./l.	Mean node of Insertion of first Adult (4-fid) Leaf*		Mean number of Leaves per plant after 6 weeks of growth*	
	2% glucose	4% glucose	2% glucose	4% glucose
0	9·33 (6)	5·50 (6)	12·33 (6)	11·67 (6)
1·0	7·40 (5)	—	10·80 (5)	—
10·0	6·20 (5)	5·0 (4)	9·80 (5)	11·25 (4)
100·0	6·40 (5)	5·40 (5)	8·20 (5)	8·20 (5)

* Number of replicate cultures in brackets.

that in the series of cultures on the 2 per cent glucose medium, at all concentrations of gibberellic acid there was a distinct increase in the rate of heteroblastic development, with the first adult (4-fid) leaf arising several nodes earlier than in the control cultures. Any suggestion that gibberellic acid is a specific 'maturity-hormone' is ruled out at once, however, by the fact that an even greater advance in the rate of heteroblastic development was obtained merely by raising the sugar concentration from 2 to 4 per cent, while furthermore, at the higher sugar concentration, gibberellic acid had no appreciable effect on leaf segmentation.

The results of the present work are certainly opposed to the view that gibberellic acid functions as a youth hormone. The undoubted effects of gibberellic acid on heteroblastic development in the investigations of various authors are more likely secondary consequences of the changes in growth rate, which so frequently follow a supply of this substance. An explanation along these lines has the advantage of accounting for the apparently contradictory results obtained with different species. Thus, the increase in growth vigour following on the supply of gibberellic acid might be expected to increase the rate of heteroblastic development in cases where the appearance of adult characteristics is dependent on the enlargement of the apical meristem[7] (as in *Marsilea* and presumably in *Eucalyptus*), while an increased utilization of carbohydrate might lead to a delay in the appearance of adult characteristics when their formation is dependent on an increasing accumulation of soluble carbohydrates in the developing organs[8] (as seems probable in ivy and *Ipomoea*).

The results of Doorenbos and of Frank and Renner can also be explained in a similar way without the necessity for postulating the existence of a youth-hormone[8]. It may, therefore, be concluded that although it is perhaps unlikely that changes in the nutrient balance will account for all types of heteroblastic development, there is no convincing evidence at present in favour of the existence of specific juvenility hormones.

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Gibberellic Acid and Nodulation of Legumes

In 1958 Thurber, Douglas and Galston[1] reported that potassium gibberellate applied to *Phaseolus vulgaris* plants had a marked reducing effect on the number of nodules formed and in some cases almost inhibited their formation. When this communication was published we were in the course of determining the effect of gibberellic acid on the nodulation of *Trifolium repens* and ultimately reported[2] that, at 25 p.p.m., gibberellic acid in agar culture had no effect on nodule numbers in this plant; and further that up to 1000 p.p.m. had no effect on the growth of *Rhizobium trifolii*. It was thus obvious that not all legumes reacted in the same way regarding nodulation. Prof. Galston explained these apparently conflicting results[3] by stating that 'this difference in nodulation response is probably related to the already known fact that different genotypes of plants presumably of varying endogenous gibberellin-levels react quite differently to identical gibberellin applications'. Further experiments which we have recently carried out with small-seeded legumes and their corresponding *Rhizobia* furnish more evidence of this variability and of the fact that reduction in nodulation, where present, is not due to the effect of the gibberellic acid on the *Rhizobia*.

The legume seeds were surface-sterilized, germinated on sterile water-agar and transferred to boiling tubes containing nitrogen-free mineral salt agar[4]. Each tube contained one plant, and there were ten replicates per treatment. One ml. of 500 p.p.m. potassium gibberellate containing 6·0 million *Rhizobia* was added to each tube. Control tubes containing no gibberellate were also set up. The plants were allowed to grow for six weeks in a cool greenhouse and then harvested.

Table 1. Effect of sodium gibberellate on the nodulation of small-seeded legumes

Species	Number of nodules		Dry weight of plant (mgm.)		
	No gibberellic	+gibberellic	No gibberellic (inoculated)	No gibberellic (uninoculated)	+gibberellic
Trifolium repens	7	8	12	7	11
T. hybridum	11	7	17	11	16
T. pratense	22	12	25	18	25
T. incarnatum	24	11	30	26	34
T. subterraneum	24	12	33	31	33
Medicago sativa	7	9	21	15	20

From Table 1 it can be seen that potassium gibberellate had no effect on the nodulation of *T. repens* and *M. sativa* whereas the nodule numbers were reduced by some 50 per cent in *T. hybridum*, *T. pratense*, *T. incarnatum* and *T. subterraneum*. In all cases the nodules formed in the presence of potassium gibberellate were effective, the dry weight of the plant being as heavy in the presence as in the absence of potassium gibberellate and heavier than the uninoculated control. In order to determine whether the reduction in nodulation, where noted, was due to the effect of potassium gibberellate on the *Rhizobia* used in the experiment or on the plant, the *Rhizobia* were grown in a liquid medium[5] containing the chemical. Turbidimetric readings were taken daily for 14 days with an 'Eel' nephelometer, and showed little or no deviation from the control. Only the readings taken at the 14-day period are shown in Table 2.

Table 2

Strain of *Rhizobia* / Conc. of gibberellic (p.p.m.)	*N/CLF* (*T. repens*)	157 (*T. hybridum*) (*T. pratense*)	*Y/SU/297* (*T. incarnatum*) (*T. subterraneum*)	*N/AH*$_2$ (*M. sativa*)
0	149×10^7	91×10^7	154×10^7	134×10^7
500	145×10^7	90×10^7	156×10^7	135×10^7
1000	148×10^7	88×10^7	158×10^7	133×10^7

From these figures it is clear that the potassium gibberellate had no effect on the growth of the *Rhizobia*. The reduction in nodulation where noted in some of the species then was due to the effect of the chemical on the plant and Prof. Galston's explanation appears to be a very likely one.

Thanks are due to Plant Protection Ltd. who supplied the potassium gibberellate for experimental purposes.

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Effect of Gibberellin on the Growth of Pollen Tubes

POLLEN grains have recently been used to study the effects of gibberellin. Kato[1] obtained a marked stimulation in germination and tube growth in *Lilium longiflorum*. In 50 mgm./l. of gibberellin the tube growth was five times that of the control during five hours. In a more extensive study Chandler[2] confirmed the growth-promoting property of gibberellic acid on pollen grains of 27 species, and 7 of these showed an increase in the percentage of germination as well as tube length.

I have investigated the effects of gibberellin (obtained through the courtesy of Dr. L. G. Nickell, Chas. Pfizer Inc., New York) alone and in combination with kinetin and 3-indoleacetic acid on the pollen grains of *Pisum sativum*. The individual effects of kinetin, 3-indoleacetic acid and boric acid were also tried.

The pollen was cultured in cavity slides by the usual hanging drop technique in a basal medium consisting of sucrose (1·0 *M*) and agar (1 per cent). The aqueous solutions of gibberellin, 3-indoleacetic acid, kinetin and boric acid in concentrations of 0·05, 0·1, 5, 10, 50, 100, 250, and 500 mgm./l. were added to the basal medium just before sowing the pollen grains. For every concentration, five sets with two cultures each were tested.

The most marked effect of gibberellin was on the tube growth. In 0·05 mgm./l. (Table 1) the tube length was 2,822μ, that is, more than seven times that of control (389 μ). At higher concentrations the growth was more than the control, but it gradually declined and at 500 mgm./l. the tubes measured only 367 μ.

Table 1

Medium	Concentration (mgm/l.)	Percentage germination	Tube length (μ)
1. *BM* (control) ..	0·0	92	389
2. *BM* + gibberellin	0·05	94	2822
3. *BM* + boric acid	100·0	96	1444
4. *BM* + kinetin ..	50·0	98	1212
5. *BM* + 3-indole acetic acid ..	50·0	89	930
6. *BM* + *kinetin + gibberellin ..	50·0	91	1029
7. *BM* + *3-indole acetic acid + gibberellin	50·0	92	897

BM = basal medium : sucrose 1·0 *M* + agar 1 per cent
The percentage of germination is based on the averages of 500 or more pollen grains, and tube lengths on the averages of 50 tubes. All cultures were maintained at 25° ± 1° C., for 24 hours.
* Concentrations of kinetin (50 mgm./l.) and 3-indoleacetic acid (50 mgm./l.) were kept constant

In 0·05 mgm./l. of gibberellin the tubes were smooth and straight, while at higher concentrations (100–500 mgm./l.), as in the control, considerable bloating (that is, broadening at tips) and bursting of pollen grains was observed.

Gibberellin had no promoting effect on germination. The percentage (92–94) remained almost constant in 0·05–250 mgm./l. of gibberellin and there was an inhibitory effect at 500 mgm./l. yielding only 59 per cent germination.

The maximum tube growth obtained with other test substances, for example, boric acid, kinetin and 3-indoleacetic acid was definitely higher than the control, but the effect of gibberellin was much more pronounced (Table 1).

Gibberellin in combination with kinetin did not bring about any improvement in tube growth. With 0·05 mgm./l. gibberellin + 50 mgm./l. kinetin the tube length was only 930 μ as compared to 2822 μ in 0·05 mgm./l. of gibberellin alone. The maximum tube length in kinetin + gibberellin measured only 1,029 μ, which was almost the same as that obtained in 50 mgm./l. of kinetin alone. Similar response was obtained with 3-indoleacetic acid + gibberellin. Thus, it is obvious that the addition of kinetin or 3-indoleacetic acid to gibberellin does not promote tube growth.

Fig. 1. Pollen tubes with two male gametes (× 160)

Fig. 2. Same, showing two and four gametes ; the arrow points to two overlapping gametes (× 160). (In both cases pollen was germinated in sucrose 1·0 *M* + agar 1 per cent + gibberellin 0·05 mgm./l.)

A notable effect of gibberellin was the stimulation of generative cell to divide frequently (96 per cent) and produce two male gametes (Fig. 1). Rarely, both the gametes divided once again resulting in four sperm cells (Fig. 2). In control, however, only 7 per cent cases of division of the generative cell were observed.

In conclusion, it may be stated that this study supports the previous observations of stimulation of pollen tube growth by gibberellin and reports for the first time, as far as I am aware, the stimulatory effect on the generative cell and male gametes.

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Action of Inhibitor-β on the Growth of *Striga* Seedlings

THE presence of a growth-inhibitory substance or substances in plant extracts, moving in paper chromatography with ammonical iso-propanol between R_F 0·55 and 0·8 was first demonstrated by Bennet-Clark and Kefford[1] who suggested the name inhibitor-β. These are now known to occur widely in plant material[2,3], and according to Varga and Ferenczy[2] the chromatographic behaviour is suggestive of a phenolic acid. Recently Bently[4] reported that the inhibitor-β zone from potato peelings contained at least six components (possibly fatty acids) and that inhibition in *Avena* straight-growth assays was caused by toxicity at greater than normal physiological

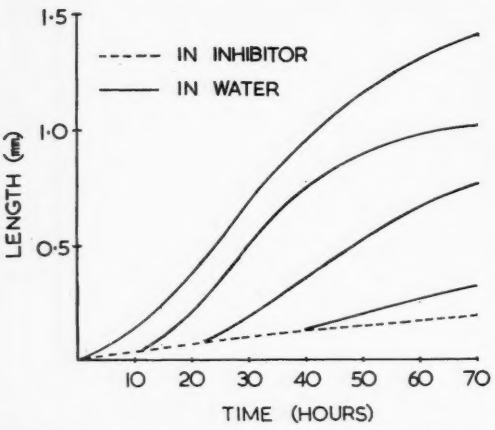

Fig. 1. Growth curves of *Striga lutea* seedlings (radicles) in water and in inhibitor-β zone; and of seedlings grown for varying periods in the inhibitor before being transferred to water.

concentrations; shown by irreversability of the effect and by loss of turgor of the sections. One of these components was shown by Taylor[5] to be azelaic acid.

Unsoaked, air dried *Striga lutea* seeds contain highly active inhibitors that move in the region of R_F 0·6 in paper chromatography, while in seeds soaked for a number of days the concentration of these inhibitors is greatly reduced. If one gram of the unsoaked seeds is extracted with 12·5 c.c. of acetone at reduced temperature (−10°C.) and 0·8 c.c. of the extract is chromatographed, the inhibitory zone being incubated with *Striga* seedlings in 2 ml. of water, a strong inhibition of the length growth of the radicles occurs (Fig. 1). The inhibition is non-toxic as is shown by the resumption of linear growth when the seedlings are transferred to distilled water after varying periods in the inhibitor solution.

The main action of the inhibitory zone is to prevent cell extension, producing a compact mass of cells in place of the normal radicle (Fig. 2). A very approximate estimation of cell numbers indicates that the effect, if any, on cell division, is small. Loss of polarity seems to occur after prolonged periods in the inhibitory solution, since the new root tip, formed on transferring to water, is frequently in a different position from the geometrical tip of the inhibited seedling. Lower concentrations reduce cell extension without causing loss of polarity. The cells formed in the presence of the inhibitor do not undergo extension on transferring to water, although general swelling occurs (Fig. 2).

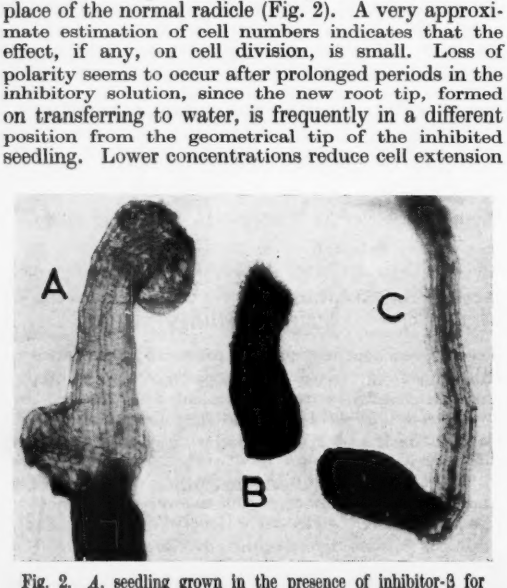

Fig. 2. *A*, seedling grown in the presence of inhibitor-β for 22 hr. and then transferred to water; *B*, seedling grown in inhibitor only; *C*, seedling grown in distilled water.

No effect on the germination of the seeds was observed but the inhibitor-β of Varga and Ferenczy[2] (from a much more concentrated extract) prevented germination of *Papaver somniferum* seeds.

The *Striga* inhibitory zone has acidic properties and the activity is not reduced by boiling for short periods in ethyl alcohol. The ultra-violet absorption spectrum of the eluted zone does not reveal a ring structure, indicating that the active substances are probably not phenolic in nature. The end absorption suggests an aliphatic acid or acids (possibly amino-acids). I am indebted to Dr. D. A. H. Taylor for preparing and interpreting the ultra-violet absorption spectrum.

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Effect of *Rhizobium* Polysaccharide on the Formation of Polygalacturonase in Lucerne and Clover

In a previous paper[1] results were presented showing that polygalacturonase is produced in associations of lucerne or clover plants and their specific bacteria. Some of the results suggested that it was formed by the plants not by the bacteria, and it was assumed that the bacteria secrete a substance which specifically induces the synthesis of polygalacturonase by the plant. It was further suggested that the active component might be a polysaccharide; the nodule bacteria used form abundant slime, and from work with the chemically related *Pneumococcus* polysaccharides it is known that such substances may exhibit a marked specificity in antigenic properties and chemical structure[2].

These assumptions have now been subjected to closer investigation. Since lucerne plants usually gave a larger response to the various treatments than did clover plants, most experiments were conducted with lucerne. Clover plants gave, however, entirely analogous results.

The bacteria were grown in shaken flasks on Dorn's A_5 medium containing sucrose as the source of carbon[3]. If mannitol was substituted for sucrose, the bacteria formed less slime and the effect on polygalacturonase formation was smaller. In order to extract water-soluble polysaccharide from the bacterial cells, suspensions of the bacteria were heated in boiling water for 15 min. The cells were separated by centrifugation and the supernatant liquid filtered through a fritted glass filter (Schott, Jena, No. 11 *G5*). This solution was tested directly. Further purification of the polysaccharide was accomplished by the procedure of Wiley and Scherp[4]. By this method a solution containg 1·5 mgm./ml. dry material with 0·8 per cent nitrogen was obtained.

As an alternative procedure, the bacteria were extracted with diethylene glycol, as recommended by Morgan[5] for a specific *Shigella* polysaccharide. The cells were centrifuged, washed in sterile water and resuspended in diethylene glycol. The suspension was

stored in a refrigerator overnight, dialysed against distilled water and centrifuged. The supernatant liquid was passed through a fritted glass filter.

The preparations obtained by the above methods were tested by adding them to Petri dishes containing aseptically grown, two-day-old seedlings of lucerne or clover. The resulting polygalacturonase activity of the liquid was measured after an additional 3–4 days, as described earlier[1]. The results are summarized in Table 1.

Table 1

Effect of various treatments on polygalacturonase (PG) formation in seedlings of Medicago sativa and Trifolium repens. PG activity expressed as per cent decrease in viscosity of a 1 per cent solution of low methoxyl pectin (Coleman and Bell, No. 7366) after 24 hr. Blanks (boiled samples) subtracted.

Series No.	Additions	PG activity lucerne plants	PG activity clover plants
1	No addition	0·6	0·7
2	Living lucerne bacteria (strain 27) ..	55·6	
3	„ clover „ (strain 226)	2·6	
4	Boiled suspension of lucerne bacteria:		
a	Whole suspension	47·1	
b	Water extract	32·2	0
c	Extracted bacteria	3·1	
5	Water extract of clover bacteria ..		8·5
6	Purified polysaccharide from lucerne bacteria:		
a	1·5 mg./dish (ca. 150 seedlings) ..	79·1	
b	0·75 mg./dish „ „	50·8	
c	0·15 mg./dish „ „	26·3	
7	Diethylene glycol extract from lucerne bacteria (dialysed) ..	48·9	

From the results given in Table 1 it is clearly seen that the nodule bacteria studied produce a water-soluble, heat-stable and non-dialysable substance which induces the formation of polygalacturonase in the corresponding host plants. Clover bacteria have no effect on lucerne plants and *vice versa*. The results indicate that the active principle is a polysaccharide.

Preliminary experiments with acid hydrolysis of the purified material and paper chromatography of the resulting solution have shown glucose to be the main constituent. A more detailed analysis is in progress.

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[PATHOLOGY

Influence of Adult Lymphoid Tissues on Polyoma-induced Tumours

Polyoma virus can induce multiple tumours in mice[1], hamsters[2] and rats[3]. To obtain tumours in a high percentage of animals, the animals must be inoculated when newly born or at a very early age. Such a requirement is not unusual for tumour-inducing viruses—the Gross leukæmia virus, for example[4]. The relationship of susceptibility to early age suggests that immunological immaturity in the host is a prerequisite for tumour induction by the virus. In this communication, preliminary results of experiments are reported which indicate that the presence of adult lymphoid tissue may be an important factor in determining the appearance and progression of virus-induced tumours.

The polyoma virus (lot *M*-11-9*a*) used in these experiments was kindly provided by Dr. E. A. Mirand (Roswell Park Memorial Institute, Buffalo, New York). It was prepared by infection of primary tissue culture of mouse embryo, and at the time of the experiments had a hæmagglutinin titre of 1:32 and infection titre of $10^{5\cdot8}$ per ml. (tested in culture of the *P*-388 line of mouse lymphoma cells). Pregnant hamsters were received two or three days before parturition from a colony (Lakeview Hamster Colony, Newfield, New Jersey) that so far has been found to be free of polyoma infection. The newly born hamsters, when 1–4 days old, were inoculated subcutaneously with 0·15 ml. of the infected tissue culture fluid. Hamster donors 2–3 months old were obtained from the same colony; and in almost all cases their sera were tested for polyoma antibodies, with negative results. Inguinal, axillary and cervical lymph nodes, spleens and thymuses were collected from the hamster donors and pressed through a steel mesh sieve. The cells were harvested and then suspended in the desired volume of Hanks's solution. The cell population of these preparations consisted of lymphocytes, lymphoblasts, reticulum cells, polynucleated leukocytes and erythrocytes. Liver cells were collected in a similar way.

New born hamsters were inoculated subcutaneously with polyoma virus before, simultaneously with, or after intraperitoneal inoculation with the lymphoid tissue suspension as indicated in Table 1. The polyoma

Table 1. Effect of Treatment with Lymphoid Cells on Tumour Induction by Polyoma Virus

Treatment	Hamsters with tumours at various intervals* 30 days	45 days	55 days	75 days
Polyoma	10/10 (100%)	11/13 (84%)	6/6 (100%)	16/17 (94%)
Polyoma followed by lymphoid cells† (0–72 hr. later)		9/9 (100%)		5/6 (83%)
Lymphoid cells† followed by polyoma (24–72 hr. later)	4/11 (36%)	8/15 (53%)	12/14 (85%)	10/11 (90%)
Liver cells† followed by polyoma		8/10 80%		

* Incidence of animals with tumours expressed as a fraction and as a percentage.
Numerator, number of hamsters with tumours; denominator, total number of hamsters in experiment.
† 50×10^6 cells.

virus induced in hamsters hæmangioendotheliomata of the cavernous type in the liver; of the solid type in the kidneys, heart and anterior mediastinum and under the skin at the site of inoculation[5]. The death of the animals was usually produced by rupture of the cavernous hæmangiomata of the liver, with resulting hæmoperitoneum, or by progressive growth of the tumour in the mediastinum, with collapse of the lung, chronic stasis and eventual heart failure. The first was the prime cause of death occurring up to 35–40 days after inoculation; the second was encountered in animals that survived this period. Each experiment was terminated at a fixed time; thus the number of animals with tumours refers to animals found dead on which a satisfactory autopsy could be performed and to animals which were killed at that time and examined. A positive score for animals with tumours includes also those cases in which small foci of tumour cells could be detected only by microscopic examination.

As shown in Table 1 the percentage of hamsters with tumours in litters inoculated with the virus alone was between 85 and 100 per cent independently of the time at which the experiments were interrupted. When polyoma was given at the same time as the adult lymphoid tissue or prior to it, no difference from the control was observed. When lymphoid tissue was inoculated prior to the virus the differences were considerable; only 4 out of 11 animals at 30 days and 8 out of 15 at 45 days had tumours; at 55 and 75 days, the number of animals bearing tumours was 12 out of 14 and 10 out of 11, respectively. Inoculation of liver cells instead of lymphoid tissue under similar conditions did not appreciably reduce the incidence of animals with tumours (8 out of 10). It is evident that the inoculation of adult lymphoid tissue prior to that of the virus considerably reduces the incidence of animals with tumours for a period after infection (45 days in these experiments). If animals are allowed to survive beyond this period, the percentage of animals with tumours is not decreased as compared with the controls, but there are fewer tumour locations per animal. The mechanism by which adult lymphoid tissue exercises this protective action can only be a matter of speculation at this moment, as too many factors are still unknown. Thus the adult lymphoid tissue could act directly by inactivation of the virus or by control and suppression of tumour cells. At this point the first hypothesis would seem more plausible, as no protection was observed when the virus was inoculated before the lymphoid tissue and as tumour cells can be transplanted into adult animals in a certain proportion of cases[5].

If the first hypothesis is correct, the fact that adult lymphoid cells did not afford complete protection can be explained on the basis that at the concentration of infectious virus units and lymphoid cells used in these experiments only a portion of the virus particles was inactivated; residual virus particles could still penetrate cells and produce tumours, but the appearance of the tumours was delayed and their number decreased. On the other hand the possibility should be kept in mind that the action of the adult lymphoid cells could be mediated through changes induced in the host. Pertinent to this point are the recent observations by Clark[6] on the changes in ingestive ability of cells in the intestinal mucosa occurring in mice and rats about 18 days after birth, and the observations of Pietra *et al.*[7] on the higher susceptibility of newly born mice to a single dose of carcinogen.

Experiments are in progress designed to answer some of the questions that these initial data suggest.

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Some Properties of Damaged Endothelial Cells

Even stationary blood remains liquid for hours in a healthy blood vessel. In a damaged vessel it clots quickly. The properties of intact vascular endothelium are difficult to study. Endothelial cells can be scraped off the lumenal surface of the aorta, for example, and some of their biological properties can be studied.

A gelatinous material was scraped off the cleaned endothelial surface of fresh human aortas and shaken in saline to give a thick suspension. It was shown histologically that this manœuvre removed the endothelium from the aorta. It was frozen and thawed twice and examined as follows:

To 0·1 ml. of citrated platelet-poor normal human plasma was added 0·1 ml. of either the endothelial cell suspension, or an optimal suspension of platelets, or an optimal suspension of brain extract as used in the 'prothrombin time' clotting system, or saline. The clotting times on recalcification in glass tubes and in silicone treated tubes were recorded. Table 1 records the average results from 6 aortas. It will be seen that the clotting time of the plasma in the presence of brain is very short in glass and is no longer in silicone-treated tubes. With the cell suspension the clotting time is shorter than the maximal acceleration produced by platelets, and is also not delayed in silicone-treated tubes, while the clotting time of the plasma containing platelets is delayed in siliconed tubes.

Table 1. The Brain-Like Activity of Endothelial Cell Fragments on Plasma Clotting Times

Citrated Platelet-Poor Human Plasma with:	Clotting Time (sec.) in glass tubes	Clotting Time (sec.) in silicone treated tubes
Brain Extract	14·2	14·0
Endothelial Fragments	42	44
Platelets	66	103
Saline	260	530

0·1 ml. of all reagents were used. Plasma and brain extract etc. as indicated were mixed. One minute later 0·25 *M* calcium chloride was added.

The endothelial cell suspension may have contained platelet material. Brain and similar tissue extracts are the only known substances normally present in the body that, when added to plasma, give a clotting time considerably faster than that given by adding platelets; and this brain-like activity is independent of contact with glass. It is concluded that aortic endothelial cell fragments have a weak brain-like accelerating effect on blood clotting.

Brain extract shares with platelet lipoid the ability to generate large amounts of thrombin when added to platelet-poor plasma, and to produce great acceleration of the Stypven clotting time (recalcified plasma plus Russell viper venom). Table 2 shows that an

Table 2. The Cephalin-Like Activity of Endothelial Cell Fragments

	1 Fibrinogen clotting time (sec.)	2 Stypven time (sec.)
Brain Extract	17·5	6
Endothelial fragments	19	9
Platelets	19·5	6
Saline	360	23

1. To 0·3 ml. of platelet-poor citrated plasma was added 0·1 ml. of brain extract etc. as indicated and finally 0·3 ml. 0·025 *M* calcium chloride. At appropriate intervals 0·1 ml. of this mixture was transferred to tubes containing 0·4 ml. of a dilute solution of fibrinogen. The shortest clotting time of any of the fibrinogen-containing tubes was recorded.

2. 0·1 ml. amounts of all materials were used. Plasma and brain extract etc. as indicated and Russell viper venom 1:10,000 were mixed and one minute later 0·025 *M* calcium chloride added and the clotting time recorded.

endothelial cell suspension is fully as active as brain extract or platelets in these two tests. It is thus rich in cephalin and much thrombin is formed in its presence.

It has been suggested that the sub-endothelial mast cells secrete a heparin-like substance which may play a part in keeping the blood liquid. All the live intact cells so far studied do not inactivate heparin[1] although intact endothelial cells have not been studied. The ability of the endothelial cell fragments to inactivate heparin was investigated as follows :

The prolongation of the clotting time caused by the addition of a given amount of heparin to a thrombin clotting system containing plasma as a source of co-factor is determined. If the addition of the cell suspension to the heparin results in a shorter thrombin clotting time it must be concluded that some of the heparin has been inactivated and is no longer 'available' to prolong the thrombin clotting time. Table 3 shows that much heparin was inactivated when the cell suspension was added. It is concluded that damaged endothelial cells can inactivate considerable quantities of heparin.

Table 3. HEPARIN INACTIVATION BY ENDOTHELIAL CELL FRAGMENTS

	Saline	Heparin 0·125 u/ml.	Heparin 0·25 u/ml.
Saline	22	51	60
Endothelial Cell Fragments	19	19	30

0·1 ml. of all reagents was used. All tubes contained platelet-poor plasma and saline or endothelial cell suspension. To these tubes was added either another volume of saline or heparin in the strength indicated. A strong thrombin solution was added and the clotting time recorded in seconds.

If endothelial cell damage or death occurs *in vivo* the two attributes of damaged endothelial cells reported above may play a vital part in thrombus formation ; these attributes are the weak brain-like activity including 'available' cephalin and the ability to inactivate heparin. Particularly may the brain-like activity be important since it initiates coagulation and is independent of any other trigger mechanism such as contact with glass.

This work was carried out during the tenure of a grant from the Medical Research Council for technical assistance, which is gratefully acknowledged.

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VIROLOGY

Infectious Ribonucleic Acid from Mouse Brains infected with Tick-borne Encephalitis Virus

INFECTIOUS ribonucleic acid has been prepared from several virus-infected tissues and from purified animal viruses[1–4] using the phenol extraction method of Gierer and Schramm[5]. Assuming that the tick-borne encephalitis virus contains a nucleic acid of ribose type, we have succeeded in preparing by the same method infectious ribonucleic acid from mouse brains infected with the *Hypr* strain of tick-borne virus isolated in Czechoslovakia. The infectivity of such preparations was about 10^3–10^5 times lower than that of virus. To be sure that the infectivity of ribonucleic acid was not caused by contamination with virus particles, the acid and virus were titrated after different treatment. The titrations were carried out in white mice by intracerebral injection of 0·03 ml. using serial ten-fold dilutions. Clarified 10 per cent (w/v) suspension of infected brains was designated as undiluted virus for convenience in comparing the infectivities of virus and ribonucleic acid. The results are shown in Table 1.

Table 1. INFECTIVITY OF TICK-BORNE ENCEPHALITIS VIRUS AND ITS RIBONUCLEIC ACID AFTER DIFFERENT TREATMENTS

Treatment	No. of exp.	LD_{50}/ml. Virus	LD_{50}/ml. RNA
None	I	$8·3 \times 10^6$	$1·3 \times 10^4$
	II	$1·3 \times 10^6$	$3·3 \times 10^3$
	III	$1·4 \times 10^6$	$6·6 \times 10^4$
Ribonuclease 45 μgm./ml. 37° C., 25 min.	I	$1·4 \times 10^5$	$< 2·1 \times 10^4$†
	II	$1·3 \times 10^6$	$< 2·1 \times 10^4$†
	III	$1·0 \times 10^6$	$< 2·1 \times 10^4$†
Heating 37° C., 25 min. (ribonuclease control)	I	—	$6·6 \times 10^3$
	II	—	$3·3 \times 10^3$
	III	—	$2·1 \times 10^4$
Neutralization with immune γ-globulin	I	$1·7 \times 10^4$	$2·1 \times 10^3$
	II	$1·0 \times 10^5$	$6·6 \times 10^3$
	III	$1·6 \times 10^5$	$1·4 \times 10^4$
Precipitation by ethanol	I	$< 1·0 \times 10^6$	$3·3 \times 10^4$
	II	$< 1·0 \times 10^6$	$5·6 \times 10^3$
	III	$1·6 \times 10$	$1·0 \times 10^4$
Heating 37° C., 4½ hr.	I	$3·3 \times 10^6$	$3·3 \times 10^3$
	II	$7·1 \times 10^7$	$<1·0 \times 10^6$
	III	$1·0 \times 10^6$	$6·6 \times 10^3$

No infectivity could be demonstrated in 0·03* (0·015†) ml. of undiluted inoculum.
—, Not done.

The infectivity of ribonucleic acid was completely destroyed by the action of ribonuclease, while that of virus was reduced significantly only in experiment 1. Control experiments have shown that ribonucleic acid is destroyed really by the enzyme and not by heat. Neutralization by specific immune horse γ-globulin lowered the titre of virus 500–12,600 times, while that of ribonucleic acid only 5–6 times. The latter remained fully infectious after precipitation with two volumes of ethanol. The same treatment destroyed the infectivity of virus. Heat inactivation did not show marked difference between ribonucleic acid and virus.

Tick-borne encephalitis virus derived from the brains of mice which died following the intracerebral injection of ribonucleic acid was identified by neutralization tests with specific immune γ-globulin. In such brains typical lesions of viral encephalitis were observed by histological examination.

The ultra-violet absorption spectra of preparations of ribonucleic acid were typical for nucleic acid. The D_{258}/D_{280} ratios were 1·94–2·14 showing that preparations of ribonucleic acid contained only very small, is any, amount of protein impurities.

The results lead us to conclude that tick-borne encephalitis virus contains ribonucleic acid which if infectious when tested by intracerebral inoculation in mice.

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BIOLOGY

A Controversial Equation in Population Ecology

An equation presented by Nicholson[1] was recently criticized by Andrewartha[2] whose comments were in turn criticized by Varley[3] and Nicholson[4]. So far, the real nature and purport of this equation does not seem to have emerged.

Nicholson[1] leads up to the equation as follows. He defines (p. 29) "the *procurement field* of the species, for the given requisite under the given conditions, as the maximum amount of space an average individual can exhaust of the given requisite in unit time when there are no new accessions of the requisite to the environment". A second characteristic is defined as follows (p. 30): "For the existence of a species it is clearly necessary that there should be a sufficient quantity of each requisite in the space of the appropriate procurement field to supply at least the minimum needs of an individual. This minimum tolerable quantity is the *maintenance quantum* of the given requisite for the species living under the prevailing conditions. When this quantity is available to an average individual, it is just sufficient to maintain the individuals and permit them to produce sufficient offspring to replace those that die." He then states (p. 31): "For equilibrium [density] of the animals, an average individual must obtain its maintenance quantum of the governing requisite [food or space] in unit time. This is possible only if the equilibrium density (G_E) of the governing requisite is such that there is one maintenance quantum (q) in the procurement field (i) of an individual. So

$$G_E = q/i.$$

It is necessary for equilibrium of the governing requisite that it should be used at the rate it is generated . . ." All this simply means that if the numbers of an animal are to remain steady then, as a primary condition, the necessary bare sufficiency of the scarcest requisite must be constantly available (cf. the once fashionable 'law of the minimum'). However, elaborating still further (p. 32), Nicholson presents the equation for "the equilibrium density (N_E) of mature animals" as:

$$N_E = (g - lg - wg)/q.$$

This is the controversial equation. According to Nicholson[4], g is "the rate of generation of the governing requisite, l the fraction of this lost to the animals due to other agencies", w "the fraction . . . wasted by the animals which scramble for it" (actually by those individuals which fail to reach maturity—see Nicholson[1]), "and q the amount of the requisite required by an animal for development to maturity". For the sake of clarity, it should be noted that q, as well as g, is a 'rate'; by definition (see above), q is the amount of requisite needed by the average individual in unit time and g the amount generated in unit time; and the time taken for the average individual to develop to maturity is the unit of time. It should also be noted carefully that, in the equation, N represents the number of *mature* animals.

For achievement of maturity, N individuals must obviously procure Q ($=Nq$) of requisite in unit time; that is:

$$N = Q/q$$

if Q is the *effective* level of supply of requisite in unit time. This is simply an equivalence, a formula for rationing which is familiar to farmers. In other words, N is just the *maximum* density possible if the average individual requires q in unit time and Q is made available in unit time. Now it is rational to regard Q as equal to $(g - lg - wg)$ and hence write:

$$N = (g - lg - wg)/q$$

but this does not alter the character of N at all. N remains the maximum possible density as described above. Why does Nicholson append the subscript E to N and call it an "equilibrium" density? Why is equivalence confused with equilibrium? Keeping in mind that supply of requisites is a coudition of enviroment, the reason is to be found in his theory of natural control of population.

The Nicholson theory[1,5] is that a population must be in a state of equilibrium with its environment and this state is brought about by competition. In the equation the available governing requisite $(g - lg)$ has been reduced to $(g - lg - wg)$ by competition. Hence, according to the theory, the equation represents the "equilibrium density". There are, however, serious flaws in this theory (cf. Milne[6,7]) and one is in the very basis of the primary assumption of equilibrium or balance. Nicholson avers[8] that "the densities of animal populations are known to bear a relation to the environmental conditions to which they are subject, and the existence of this relation shows that populations must be in a state of balance with their environments". But the relation is simply that densities become high in good conditions and low in bad. This relation undoubtedly exists. But the existence of a relation does not show (prove) that it must be one of balance. Relations other than balance can exist. The relation here is essentially one of cause (environmental conditions) and effect (population density). It is true that should population chance to rise sufficiently high, it will itself modify some of the conditions significantly (bringing another condition, intraspecific competition, into play) but the relation remains nevertheless one of cause and effect.

Returning again to the vexed equation: Varley[3] is wrong in holding that w is a function of N; w is obviously a function of $(N + N')$, where N' is some unknown number of individuals that died at various unknown stages of development short of maturity; in other words, competition is not functionally involved in the equation. It is therefore not an "equation for intraspecific competition" (see Andrewartha[2]). Nor is it an equation for "equilibrium density" as Nicholson[1,4] claims and Varley[3] accepts. It is merely an equation showing the maximum density possible with an amount of requisite which has been determined by its rate of generation and rate of loss through competition and other agencies. It contributes nothing new to, or useful in, the problem of natural control.

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It is a fundamental rule of physical equations that the two sides must balance in terms of the dimensions ascribed to the magnitudes that they represent[1]. The same rule should hold when we try to apply physical equations to biological processes.

Varley[2], in discussing Nicholson's equation:

$$N_E = \frac{g - lg - wg}{q}$$

gives the following definition of terms, to which I have ascribed dimensions, using M, L and T in their usual sense.

N_E = equilibrium population density: L^{-2} for terrestrial animals

g = food supply: if this is presumed to mean 'on unit area', $ML^{-2}T^{-1}$

l = fraction of food lost to extraneous factors: (numeric)

w = fraction of food wasted by surplus individuals that fail to mature: (numeric)

q = quantity of food required to complete development: M

This makes the equation balance, but also makes it almost useless in practice since only in very special circumstances, such as the development of an egg, do situations occur in Nature in which there is a discrete food supply that is not added to during the life of the population.

Nicholson[3] gives the same definition of N_E, and effectively the same of q, but defines g as the rate of generation of the governing requisite, and l and w as fractions of this. In the case argued by Varley, where the governing requisite is food, the dimensions of the equation then become:

$$[L^{-2}] = [ML^{-2}T^{-1}]/[M] = [L^{-2}T^{-1}]$$

which is absurd. There is confusion between the rate of supply of food and the total quantity required. (Both may be important to the animal, since it needs not only enough food but also enough at the right time.)

When one turns to Nicholson's original paper[4], one finds the same definitions for N_E, g, l and w, but q is said to be the maintenance quantum in the procurement field (i) of an individual, so that:

$$G_E = q/i$$

where G_E is the equilibrium density of the governing requisite. This would seem to make the dimensions of q to be M, as before, so that the equation still does not balance. It is difficult to see how equations that fail to pass the most elementary kind of dimensional check can be of any help in the study of ecology.

I have had the benefit of discussing the method of dimensions with Mr. G. R. Noakes.

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Milne gives his case away at two points in his argument. His first difficulty is largely semantic. He prefers the term 'maximum density' where Nicholson used 'equilibrium density'. In population experiments begun at low densities, where the numbers rise asymptotically to a maximum, both terms are equally applicable. However, populations often overshoot the equilibrium denstity and settle down to this value afterwards; or they may be begun experimentally at levels above the equilibrium. Nicholson's more general term is applicable to all these cases, whereas Milne's is not.

Nicholson's experiments have shown how equilibrium population densities vary with the conditions, and surely all would agree with Milne that this relationship is one of cause and effect. This in no way renders Nicholson's terms balance or equilibrium less suitable.

In his last paragraph Milne fails to see that my general statement that w must be treated as a variable which is a function of the population density N does not conflict with the more specific relationship which he proposes—especially as his N' must itself be a function of N. Although it is critical to the understanding of the behaviour of crowded populations, the actual form of the relationship remains undiscovered. Finally, if Nicholson's formula is, as Milne claims, an elaboration of one already used by farmers, it seems perverse to suggest that it is useless to those ecologists studying single species cultures.

Yapp's communication raises the important question of the dimensions to be attached to the terms in Nicholson's equation and I am very glad to have the opportunity of clarifying the matter. Nicholson (*l.c.*, p. 30) says that the maintenance quantum q represents 'an average of the varied amounts of the given requisite obtained by different individuals *in unit time* ehen living under equilibrium conditions' (my italics). Andrewartha in *Nature* of January 17 incorrectly redefined q as 'the amount [of the requisite] required by one individual to complete its life cycle', and I regret that in our replies in *Nature* of March 28 both Nicholson and I used similar definitions and wrongly omitted the vital words "in unit time". Nicholson's unfortunate choice of the terms "maintenance quantum" and 'procurement field' for what are (as Milne correctly says) rates of feeding and of searching is, I consider, partly to blame for the loose way in which the terms have been discussed.

Since the dimensions of q, g and i all include T^{-1} Yapp's difficulty disappears. The time unit might, as Milne suggests, be taken as the time for an average individual to develop to maturity, but must in any event be the same for each of the terms.

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Cause of Wear in Sheeps' Teeth

In New Zealand the problem of wear in sheep's teeth is acute on some pastures, and as a result of detailed investigations, Barnicoat[1] suggested that wear is due to the abrasive action of the fibre in herbage although he could not correlate the amount of fibre in herbage with the extent of wear. While Barnicoat (p. 586) found no evidence to justify further studies on the mineral content of herbage we, on the other hand, believe that such studies may provide the clue that leads to an understanding of wear.

Our approach is based on the knowledge that opal-phytoliths are secreted within pasture and fodder plants in varying amounts. In fact, practically all the silicon in mature plants is in the form of these solid mineral particles[2]. Furthermore, we have obtained opal-phytoliths in the fæces of sheep and find many of them to be in a fractured condition. The fact that opal is a relatively hard mineral leads to the idea that opal-phytoliths in pasture and fodder plants are largely responsible for wear in sheep's teeth.

We have carried out relative hardness tests by two methods on the molars and incisors of the sheep and on opal. The teeth examined were from a four-year-old sheep which had been fed on pasture. They showed considerable cratering of all molars and rather less

cratering of the incisors as a result of differential wear of the several tissues of the teeth. The first hardness test is based on the resistance to scratching by a standard set of minerals. It showed that the enamel was the hardest of the teeth tissues being between 4·5 and 5·0 on Moh's scale of hardness (range = 1–10). The cratered central portions of the tooth consisting of remnant dentine and the tissues of the pulp chamber were significantly softer and between 3·5 and 4·0. Opal has a hardness of 5·5–6·5 on Moh's scale.

The second hardness test is based on the resistance of a material to indentation by an indenter of fixed geometry under a static load. Tests on our experimental material were carried out by the Commonwealth Bureau of Dental Standards (Australia) using a Leitz-Durimet micro-hardness tester with a Knoop diamond and a load of 100 gm. Hardness measurements made at intervals of 0·1 mm. on polished transverse sections of the teeth are summarized in Table 1. We found that individual opal-phytoliths (from oats) gave a Knoop hardness of 590–610 while opal ranges from 450 to 620.

Table 1. Knoop Hardness Values of Sheep's Teeth

Teeth tissue	Molar: Buccal to lingual surfaces	Incisor: Labial to lingual surfaces
Enamel	382 ↓ 270	326 ↓ 282
Dentine	76 ↓	74 ↓
Pulp chamber	33 ↓ 79	< 33 ↓ 62
Enamel	274 ↓ 366	240 ↓ 317

Since opal is harder than the tissues of sheep's teeth one can scarcely avoid the conclusion that opal-phytoliths are a cause of wear in sheep's teeth. If we assumed that a sheep eats 3 lb. a day of dry matter containing 2 per cent amorphous silica the intake of opal-phytoliths will be of the order of 22 lb. per annum. This is an appreciable amount of abrasive material, and some of it is crushed between the crowns of the incisors and the pad while cropping the herbage. Much of it passes under pressure between the crowns of the upper and lower molars more than once due to rubbing and grinding during the uncomplicated lateral jaw movement of the ruminant[3]. The calculated intake of 22 lb. per annum of opal-phytoliths can vary with the content of opal-phytoliths in different types of herbage. When the intake is higher, and it can be twice as high in some gramineous species, the rate of wear will increase; when lower as in herbaceous species the rate will decrease.

While attrition by opal-phytoliths in pasture and fodder plants may be a major cause of wear in sheep's teeth, we recognize that some attrition could be due to opal-phytoliths and other hard minerals contained in soil[4–6]. The sheep can obtain these mineral particles either directly from the soil or indirectly from pasture where they exist as adventitious material on the external surfaces of plants. We have observed occasional angular fragments of quartz (Moh hardness = 7, Knoop hardness = 710–790) as well as abundant angular fragments of opal-phytoliths in sheep's fæces. Many of these fragments are undoubtedly produced during biting and mastication, and indicate that the process of indentation of teeth tissues is to be added to those of rubbing and grinding.

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Sectioning Large Heavily Sclerotized Whole Insects

The following account shows how to obtain much-improved sections of large heavily sclerotized whole insects. The method should be applicable to many other kinds of animals which present similar problems. Use is made of a fixing, dehydrating, and clearing agent, tetrahydrofuran, which produces a minimum of tissue hardening. It is highly volatile thus permitting rapid removal in the paraffin oven, dissolves paraffin and celloidin. Tetrahydrofuran (furan) has been used by other workers with good results[1,2,3]. Paraffin infiltration is done in a partial vacuum. The blocks may be soaked 24 hr. or longer in either 'Mollifex' (B.D.H.) or in water (preferably with a small amount of detergent) or in 75 per cent alcohol. Before sectioning, the blocks in the soaking medium, if such is used, are cooled to approximately 15°C. Better than average sections are obtained.

What really permits of superior sections is the application of a special kind of adhesive tape to the block before each section is cut. By so doing the section with all the tissues in place is firmly held to the adhesive tape, shows no compression, therefore requires no further spreading. The section can be applied to a slide and the tape removed easily. Tape can also be used to obtain serial sections of celloidin embedded material. The idea of using tape to hold sections was first reported by Palmgren[4] using 'Scotch' cellulose tape. But the tape to be described here is much easier to use.

Paraffin.—We have fixed, embedded and sectioned whole *Cecropia* silk worm pupæ.

The tissue is fixed as desired. We used 1 part chloroform, 6 parts furan and 1 part glacial acetic acid for 1 hr. The tissue is then transferred to pure furan for 1 hr.; then to furan and 2 per cent celloidin for 1 hr. The latter may be omitted but should be replaced with a 1-hr. period in furan and paraffin 50:50 at 60°C. The tissue is then placed in pure tissuemat at 60°C. for 3 hr. The paraffin is changed and the pressure is lowered to 20 in. of mercury. A few bubbles of furan slowly escape from the tissue. The tissue is left for 3 hr. The paraffin is again changed and the partial vacuum increased to 25 in. of mercury and the tissue left for 12–24 hr. The tissue

is embedded in tissuemat (Fisher Sci. Co., Ltd.) at 56–58°C.; other similar paraffin as well as ester wax (B.D.H.) gives equally good results.

The block is trimmed and mounted for sectioning. It is cut normally until the tissue is exposed, then placed in 'Mollifex' for 24 hr. For the last 2 hr. it is kept in the 'Mollifex' at 5°C. in a refrigerator. The block is then removed and sectioned. We section in a draft of air at 15–18°C. A sharp evenly honed knife must be used to prevent waves appearing in the sections. The soaking gives the best section of cuticle; the enclosed tissue is sometimes poorly cut. If the enclosed tissue is of prime importance the soaking should be omitted. The cuticle will section well but will show some cracks. A foot or so of 'Scotch' tape No. 810 (permanent mending tape Minnesota Mining and Manufacturing Co., London, Ontario) is cut from a roll of a width which just covers the block. One end, an inch or so in, is applied to the block and pressed evenly with the finger. The knife is then drawn through the block rapidly, taking care not to cut the loose tape at each end of the block. The tape with section firmly attached comes free. With experience, serial sections can be cut rapidly. The uncompressed section is permanently preserved, can be handled and stored easily and studied with the microscope for gross morphological detail.

The sections are stained and mounted as follows: a clean slide is lightly albuminized and a few drops of water applied. A section, or a row of sections, is cut from the length of tape and placed sections down on the water. The slide is then blotted and put on a warmer to dry with a heavy weight on a blotting paper over the section on the slide. In a few hours the section is dry. It is then placed in furan, and the tape, adhesive and paraffin dissolves off in 30 min. The section adheres to the slide. The tape can also be removed by placing the slide in chloroform. In 2–5 min. the backing of the tape, an acetate, shrivels and by grasping one corner with forceps the backing can be removed leaving the adhesive and the section on the slide. Xylol removes the adhesive and the paraffin in about 30 min. To ensure the complete retention of the section during staining the slide is placed from xylol or furan to 1 per cent celloidin in ether:alcohol for 30 sec., then removed and dried. From here the normal staining procedures are used. After staining, the celloidin can be removed but always with some risk of losing chitinous parts.

A more rapid and more satisfactory method of affixing the sections is possible. To a slide coated with albumin add a few drops of 2 per cent celloidin in methyl benzoate or of 2 per cent celloidin in absolute alcohol. Place the tape, section side down, on the fluid and blot; then apply firm pressure over all the section to squeeze out excess fluid and to ensure close contact of the tissue with the albumin and slide. Neither methyl benzoate nor alcohol dissolves paraffin, so the tissue held in paraffin is not displaced or altered when pressure is applied. After 30 sec. to 1 min. transfer the slide to pure chloroform. The celloidin is fixed and holds the section to the slide. The backing of the tape shrivels in the chloroform and in 2–5 min. can be stripped off. The adhesive and paraffin are then removed in xylol in 30 min.

Another tape which we find superior to 'Scotch' tape No. 810 is 'Mystic Kylon' tape No. 6400. (Mystik Tape Sales (Canada) Ltd., Toronto 13.) It has a very flexible polymer backing and a very tacky adhesive. Sections 1 μ thick can be cut with the 'Mystic' tape. To affix sections to the slide water on albumin is not satisfactory, but the use of 2 per cent celloidin in methyl benzoate as described above gives excellent results. If the odour of methyl benzoate is too objectionable, a mixture of 2 per cent celloidin in acetone/ether 50:50 may be used instead. The acetone will not dissolve the paraffin or the tape adhesive and the diluted ether only does so slowly. This solution gives ample time to press the section to the slide without any tissue displacement. It is recommended that at first the tissue-pressing operation be done under a stereoscopic microscope so that all excess fluid and bubbles are removed from under the tissue and displacement can be watched for. After staining, some of the excess celloidin can be removed by dipping the slides in ether alcohol 50:50 once or twice, but there is risk of losing parts of the cuticle. Carboxylol or butyl alcohol, which will not dissolve celloidin, must be used between 95 per cent alcohol and xylol. Any treatment with absolute alcohol of sections held by celloidin will cause the sections to drop off.

Celloidin.—Tissue infiltrated and embedded in celloidin in a standard fashion gives excellent serial sections if the blocks are stored in terpineol. The block is blotted just before applying the tape to the block; then the section is cut. No further blotting is required. Sections can be cut from 5 μ. To prevent large pieces of tissue from cracking when exposed to air the sections should be fastened to a slide as soon as possible. The rapid method of fastening the sections with celloidin in absolute alcohol, or in methyl benzoate for 'Scotch' tape No. 810 or celloidin in methyl benzoate or in ether/acetone with 'Mystic' tape is excellent. Chloroform is again used to remove the tape. If 'Mystic' tape is used, the sections on the tape can be stored in butyl alcohol and later applied to a slide.

I am indebted to Mr. Uno Paim who suggested the use of furan, Mr. Joseph Habowsky who tested the method on many sections and suggested improvement and Dr. K. G. Davey for his support. Thanks are also due to Miss V. C. Jackson of the Department of Zoology, who supplied the first celloidin blocks to be sectioned. Prof. V. B. Wigglesworth saw some of the results and read the manuscript; his comments are much appreciated.

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Aug. 7.

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Priapulus abyssorum, New Species, the first Abyssal Priapulid

A single example of *Priapulus* was collected on November 27, 1958 by the R.V. *Vema* from a depth of 5680–5690 m. (corrected) at the southern end of the Mid-America Trench. Besides being a new species and the first abyssal record for the genus, this specimen represents the first priapulid collected from off the west coast of Central America. The specimen is a 'missing link' in what was considered a classic case of bipolar distribution for a benthic genus. The bipolarity of *Priapulus* is discussed in detail by Ekman[1]. He states 'Here the present day discontinuity may be regarded as proved'. The species *Priapulus horridus* Theéle (1911) from Argentina customarily has been dismissed from the problem on account of its distinc-

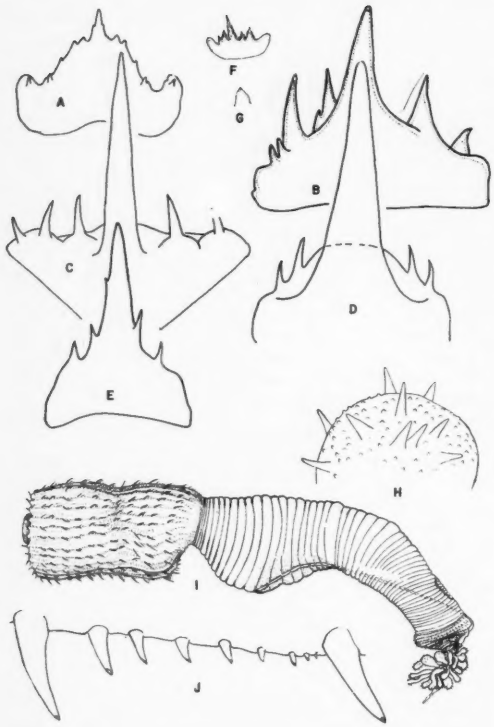

Fig. 1. *Priapulus abyssorum*, new species, *A-G* mouth teeth; *A*, first pentagon; *B*, second pentagon; *C*, third pentagon; *D*, fourth pentagon; *E*, fifth pentagon; *F*, tooth twelve rows from fifth pentagon; *G*, tooth eight rows from F; *H*, respiratory vesicle; *I*, lateral view of holotype; *J*, lateral view of spines on intovert.

tive and unusual morphology. Possibly *horridus* belongs in another genus. The same can not be said for the species reported here. The animal is allied so closely to *Priapulus tuberculatospinosus* Baird, the species characterizing the Antarctic, that some may doubt whether the two are distinct species. A positive decision can not be reached without additional specimens of various sizes. Nevertheless, with this record the tropical submergence of *Priapulus* may be considered a fact and its 'bipolarity' only a matter of historical interest.

It is a pleasure to acknowledge the kindness of Prof. Dr. Karl Lang of the Swedish State Museum who sent two of Theéle's most important papers[2] on *Priapulus* and thus enabled this study to be brought to a conclusion.

Description: Introvert one-third the total body length, provided with sharp chitinous spines arranged in twenty-five rows. Behind each major spine in a row are four to eight successively smaller spines. Trunk dull, with about 50 annulations. Caudal appendage about one-seventh the length of body and with about 30 unbranched respiratory vesicles which bear spines. Posterior extremity of trunk annulate and lacking warts. Mouth with distinctive teeth arranged in pentagons. Teeth of first pentagon with very short spines, tooth about one-third the width of tooth of second pentagon. Teeth of second pentagon the largest, each subequal in size, and with a long narrow medial spine. Teeth of third pentagon similar to second. Teeth of fourth pentagon similar to third but with smaller lateral spines, each tooth similar to other in size. Teeth of twelfth row from fourth pentagon teeth small and with serrated spines. Teeth eight rows beyond consist only of projections of the œsophagial chitin with a single apical spine. Gonads ripe and paired.

Measurements: Length introvert 8·00 mm., trunk 11·5 mm., caudal appendage 2·5 mm.

Type locality: Lamont Geological Observatory Biotrawl No. 143, VEMA-15, latitude 12° 11′ North, longitude 89° 34′ West, at 5680–5690 m. depth (corrected from sonic fathoms), from the floor of the Mid-America Trench, November 27, 1958, one holotype in the collections of the Lamont Geological Observatory.

Affinities: This species is closely allied to *P. tuberculatospinosus* Baird. It differs from that species in having all teeth of the fourth pentagon subequal in size and in lacking warts from the trunk extremity.

Collections made on *Vema*-15 Cruise through contract with the Office of Naval Research, the Bureau of Ships, U.S. Navy, and the National Science Foundation. Laboratory work was supported by a grant from the Rockefeller Foundation.

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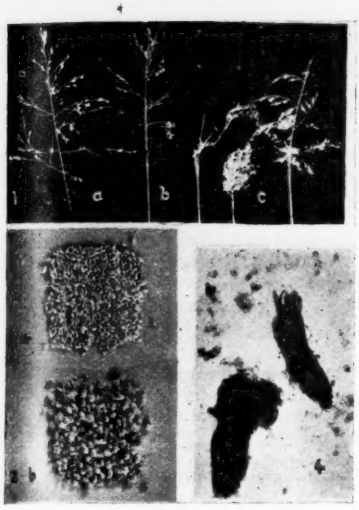
Floral Malformation in *Panicum antidotale* caused by a Species of *Eriophyses*

DURING March–April in 1958 and 1959 a peculiar malformation of flowers (Fig. 1) of blue panic (*Panicum antidotale* Retz.) was observed at the Institute of Agriculture, Anand. Individual flowers were malformed into false grains similar to smut sori (Fig. 2). When few flowers in an ear-head are infected and malformed, the disease is difficult to be noticed in the field (Fig. 1*b*). However, when the whole inflorescence is involved, it becomes quite prominent (Fig. 1*c*). The disease is quite prevalent in the vicinity of Anand. Malformation of panicles in mango[1] (*Mangifera indica* L.) more or less similar to above has been described. However, in case of mango malformation, invariably, whole inflorescence is involved and no fertile flowers are ever borne on such panicles[1] and gives the appearance of witch's broom[2].

A young affected flower of blue panic grass when dissected and examined under the microscope revealed no malformation in the accessory whorls of the flower. The malformation is confined only to essential (reproductive) whorls (Fig. 3). The filaments of the anther lobes are flattened, opened out and present leaf-like appearance (Fig. 3*a*). The anther lobes are malformed, curved inwards and show single irregular layer of one cell thickness. No pollen grains are observed in cases examined. The lower part of style is also fused with ovary (Fig. $3s_1$) while the stigma becomes enlarged and deformed (Fig. 3*s*). The free style below the stigma becomes thicker than the style in a normal flower (Fig. $3s_1$). The fused ovary is excessively enlarged (Fig. 3*O*). The ovary of an affected flower along with fused filaments and style present a compact spherical appearance whereas the general outline of a healthy flower is oval or lanceolate.



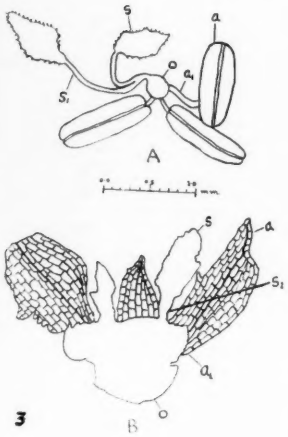


Fig. 1. (*a*) Healthy earhead of blue panic ; (*b*) part of the inflorescence is affected ; (*c*) almost whole inflorescence is malformed.
Fig. 2. (*a*) Healthy seeds of blue panic ; (*b*) malformed ovaries.
Fig. 3. (*A*) Essential whorls of a healthy flower ; (*B*) essential whorls of a malformed flower. (*a*) anther ; (a_1) filament of anther ; (*S*) stigma ; (S_1) style ; (*O*) ovary.
Fig. 4. Photomicrograph of a species of *Eriophyses*.
Figs. 1–4

Microscopic examination of the deformed flower revealed numerous disease-inciting *Eriophyses* sp. (Fig. 4) which is also considered to be the disease inciting mites in case of mango malformation[1]. Although the mites in mango are numerous in meristematic regions and tender portions of the peduncles, those in blue panic are restricted only in floral parts. Examination of healthy flowers and other portions of the inflorescence showed none of them. In later stages of malformed 'flowers' the various phases of the developing mite were observed. Furthermore, it was noticed that the enlargement was mainly responsible due to cell enlargement.

We wish to express our thanks to Messrs H. A. Joshi and B. G. Jaisani of Department of Botany for rendering help in botanical description and camera lucida drawings of healthy and malformed flowers.

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A 40-Chromosome Variant in *Solanum nigrum* L.

The literature on *Solanum nigrum* L., indicates that it is a complex of several distinct species[1].

A plant from the small natural population of hexaploid *nigrum*, growing in the Botanical Gardens of the University of Allahabad, revealed more often $6_{IV} + 8_{II}$ ($n = 20$) as shown in Fig. 1, one bivalent remaining attached to the nucleolus. In one or two cases an octovalent was also observed. Such a plant can only originate from an haploid ($n = 36$) plant as is explained herewith.

The natural haploid *nigrum* ($n = 36$) with $^2 12_{II} + 12_I$ is poly-haploid in the true sense where the first meiotic division will separate 12_{II} into 12 + 12 chromosomes going to two poles and 12_I separating at random, the number being anywhere between 0 and 12. As a result of such separation, if a dyad with 12 + 8 (20) chromosomes undergoes a normal second division, which may or may not form a cell wall, then in the former case tetrad with 4×20 chromosomes, and in the latter case a polyploid dyad with 40 chromosomes, that is, $2 \times (12 + 8)$, will form. Both cases may give rise to a progeny with 40 chromosomes : in the former case if a male nucleus having originated in the same fashion with 20 (12 + 8) chromosomes enters to fertilize an egg nucleus, and in the latter by the process of female parthenogenesis. These might hold good in the present observation where 8_{II} have been observed pairing perfectly and the remaining 24 chromosomes pair to form quadrivalents, and in few cases octovalents and quadrivalents. Though Love and Suneson[3] have shown in *Triticum-Agropyron* hybrids that regular meiosis is not essential for the production of viable gametes, the

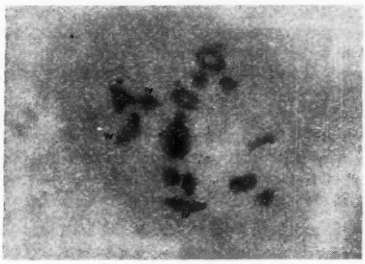

Fig. 1

second case is more plausible because: (1) the egg can survive unbalance to a greater extent than the pollen grain (2) female parthenogenesis has been reported to occur in *Solanum* so that the haploid ($n = 36$) under consideration itself has been interpreted as originating parthenogenetically[4] from the auto-allohexaploid *nigrum*; and (3) the formation of perfect 8_{II} is easier to interpret on the basis of duplication of the same chromosomes rather than the pollen grain with the prototype chromosomes entering to fertilize the egg nucleus.

The species status of the variant will be an interesting study.

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High Levels of Phosphorus and Die-Back in Yellow Lupins

In a rotation experiment[1] with conifers and green crops in a Forestry Commission nursery on very acid heathland soil near Wareham, Dorset, yellow lupins *Lupinus luteus* grown with fertilizer have often shown signs of die-back in the older leaves, occasionally with more extensive damage leading to the death of the whole plant.

In the early stages of the symptoms the leaflets become incurved, and death of the tissue starts near the tip of the leaves and proceeds inwards from the edges. In the final stages the leaflets turn grey and drop. Sometimes translucent patches and small brown spots have been noticed before the more severe symptoms set in.

Samples of plants, both healthy and affected, were obtained for chemical analysis from a number of plots at Wareham nursery that had been treated with fertilizer. The percentages of nitrogen and potassium seemed normal for young plants, but the phosphorus values in those affected were abnormally high, reaching 2·2 per cent phosphorus in dry matter for the most severely affected plants (Table 1).

Table 1. Phosphorus Contents of Healthy and Affected Young Lupins, Wareham, 1958

	dry weight per plant (gm.)	Phosphorus in dry matter of plant (per cent)
Plant, healthy	0·7	0·6
Leaflets with brown spots ..	0·5	1·7
Leaflets with severe die-back	0·4	2·2

The possibility of phosphorus toxicity was examined in a pot experiment at Rothamsted using Wareham soil and testing five levels of application of monocalcium phosphate (0–64 mgm. phosphorus per plant). Monocalcium phosphate was used because it is the essential constituent of superphosphate which had been applied in the nursery. All pots received basal dressings of nitrogen, potassium, calcium and magnesium. Table 2 shows the visual score for damage, dry-weight and phosphorus content of the plant.

Table 2. Pot Experiment with Wareham Soil at Rothamsted, 1958

Phosphorus applied per plant (mgm.)	Visual 'score' for amount of die-back	Dry weight per plant (gm.)	Phosphorus in dry matter of plant (per cent)
none	0	1·6	0·16
8	1	1·7	0·53
16	3	1·6	1·10
32	6	1·2	1·76
64	10	0·7	1·98

The degree of damage increased with the amounts of phosphorus applied and with the phosphorus contents of the plants. At the highest rate of phosphorus with a visual 'score' for damage of 10, all except two or three of the youngest leaves were dead or dying.

Damage is slight when the phosphorus content of the leaves is less than 1 per cent, but so severe at 2 per cent that the lupin plants ultimately die. The results of this pot experiment confirm the suggestion that the plants grown at Wareham nursery were suffering from excess of phosphorus.

At another nursery, near Oxford, yellow lupins grew normally when given the same fertilizer treatment as at Wareham. The soil at Oxford, however, was a sandy loam and less acid. Although the differences in the soils of the two nurseries may account in part for the differences in the effect of application of phosphorus fertilizer, sitka spruce and other conifer seedlings not only show no damage on the Wareham soil but growth is increased by superphosphate applied at comparable rates. Sitka spruce seedlings take up less phosphorus than the lupins; the level of phosphorus in the plant is only about 0·3 per cent, and this is little affected by varying the rates of superphosphate. Rossiter in Australia[2,3] found that subterranean clover and oats grown on certain sandy soils had high concentrations of phosphorus and that the plants were also damaged.

As our results show striking differences, not previously reported for the United Kingdom, between the behaviour of different crops and soils, more detailed experiments are planned.

The experiments were part of an investigation done in collaboration with the Research Branch of the Forestry Commission.

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A Quick Method for Extracting Plant Embryos from Certain Types of Seed

One of the most difficult problems in the culture of plant embryos is to find a satisfactory way of extracting the embryo from the seed without damage and in such a manner that both embryo and culture medium remain sterile. This is particularly difficult if the seed coat is very hard, and usually dissection under sterile conditions is the standard method employed[1,2]. The following technique was originally evolved to provide an easy method for extraction of mature *Pœonia* embryos from their seeds, and has since been used in the removal of embryos from other hard-coated seeds.

The dried seeds are first soaked until fully hydrated. They are then immersed for 30 min. in concentrated sulphuric acid, thoroughly washed in running water for at least 1 hr., and incubated in water overnight at 35°C. After incubation they are surface dried. A gentle pressure on the seed between the fingers will cause the embryo to be extruded through the micropyle. The embryo may thus be squeezed directly on to the agar culture slope in a few seconds with virtually no danger from contamination from the atmosphere. The embryos subjected to this treatment are viable in every way and commence to develop almost immediately.

The extraction technique can also be used on mature seeds of *Nuphar* and *Nymphœa* species. The embryos of these plants are contained within the seed in a small sphere of endosperm which lies just beneath the operculum and is embedded in perispermic tissue[3]. After incubation the operculum is readily removed and gentle pressure causes the entire endosperm sphere to emerge. The embryo can be easily extracted from this without damage as the endosperm tissue is very soft.

The time necessary in acid will vary according to the species and must be initially determined for each. It is largely dependent upon the size of the seed and the nature of the testa. Generally, the larger the seed, the longer the period that is necessary. The time spent in running water should not be reduced as this may affect the subsequent viability of the embryos.

This technique is particularly useful if large numbers of intact, viable embryos are needed, free from contamination, for use in physiological and other experimental work.

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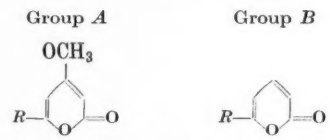
Phylogeny of the Genus *Aniba* Aubl.—A Comparative Morphological and Chemical Observation

EVOLUTIONARY progress in the plant kingdom is usually evaluated on a structural basis; only a few attempts have been made to utilize chemical facts in phylogenetic reasoning. The concomitant changes in the morphology and chemistry of *Eucalyptus*[1] and *Callitris*[2] species were studied by Baker and Smith. McNair correlated the evolutionary status of plant families with the chemistry of their constituents[3,4].

We believe that observations of this kind could be extended in the light of our present knowledge of biosynthetic pathways and present an example which shows the results that can be expected from efforts of this kind.

A number of α-pyrones are known to occur in several species of the plant genus *Aniba* Aubl. (family Lauraceae). Chemically, these can be divided into two groups, according to the presence or absence of a methoxyl group at carbon atom 4 of the pyrone ring. (Table 1).

Table 1



R	Group A compounds	Group B compounds
Phenyl	4-methoxyphenylcoumalin (5)	phenylcoumalin (8)
Piperonyl	4-methoxyparacotoin (6)	paracotoin (8)
β-Styryl	5,6-dehydrokavain (7)	
β-Pyridyl	anibine	

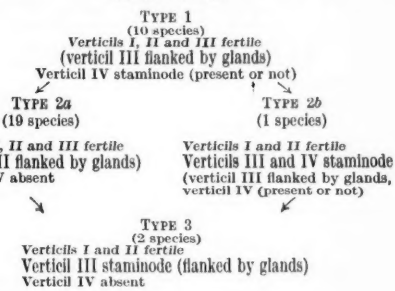
Figures in brackets are reference numbers.

Compounds of the group *A* type have been encountered in *Aniba rosaeodora* Ducke, *A. Duckei* Kostermans and *A. firmula* (Nees *et* Mart.) Mez, whereas type *B* is represented in *A. coto* (Rusby) Kostermans and *A. pseudocoto* (Rusby) Kostermans. We have recently suggested that this distinction may possibly have a phylogenetic significance[5].

Acetic acid units are admittedly the biosynthetic building blocks of polyketomethylene chains and their cyclization products. Experiments with isotopically labelled acetate support this theory and many examples are known of natural compounds with the oxygen atoms in the required positions. The above group *A* compounds are typical examples. We have therefore suggested that in the group *B* type the oxygen function at carbon atom 4 has been lost, assigning to those species which produce such substances a more recent origin in the evolutionary history of the genus. (Birch and Donovan[9] had already included phenylcoumalin among the compounds originated through the linkage of acetate units.)

In order to verify the validity of this assumption on conventional morphological grounds, a comparative analysis of the floral verticils of the known species of *Aniba* has been undertaken, based on the classical phylogenetic concept of gradual reduction and suppression of whorls in the evolution from primitive to the more recent forms. Since in *Aniba* perigonium and gynæcium show practically no variability, observations were concentrated on the androecium.

Table 2. PHYLOGENETIC DEVELOPMENT IN THE GENUS *Aniba* AUBL. BASED ON THE GRADUAL REDUCTION AND SUPPRESSION OF VERTICILS IN THE ANDROECIUM



In Table 2 it is shown how in *Aniba* the androecium, starting from a primitive type 1 with three fertile and one sterile verticils, developed through two parallel suppressive and reductive routes (types 2*a* and 2*b*) to type 3, with only two fertile and one sterile verticils.

In this scheme, *A. rosaeodora*, *A. duckei* and *A. firmula* (the species known to contain type *A* compounds) fall into class 1. *A. coto* and *A. pseudocoto* are to be placed in class 2*a*, the latter species representing already a transition form to class 3, since the minute lateral locules of the anthers of the inner whorl of fertile stamens (III) already indicate a tendency toward the staminode condition (as is also found in class 2*b*).

In this way it appears that the morphological and chemical evidence support each other. Not only is the chemical reasoning in accordance with the structural criterion, but even lends to it a new and significant basis, since the biosynthetic pathway clearly shows the only possible direction in which evolution could have taken place.

Financial aid from the Conselho Nacional de Pesquisas is gratefully acknowledged. This communication is one of a series on the chemistry of rosewood[5], we should like to dedicate it to the memory of the late Adolfo Ducke.

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Skin Homografts in Parabiotic Mice

The failure to obtain successful homografts in non-related individuals of the same species is due to antigenic differences occurring between the donor and the recipient on the basis of which the specific immunological reaction, transplantation immunity, results. Tissue incompatibility can be overcome either by physical, chemical or biological treatment of the transplant itself diminishing its antigenic diversity in relation to the recipient or by changes in the immunological reactivity of the recipient against the antigens of the transplanted tissues. Hitherto the best method of opposing tissue incompatibility and theoretically the most significant is the experimentally acquired tolerance of the foreign antigens elicited by the action of these antigens on the organism in its early ontogenic stages. This can be accomplished by means of intra-embryonal injections of homologous cells[1], of injections in new-born animals in the 'adaptative period'[2], by experimental parabiosis in birds[3] and by exsanguination blood transfusion performed shortly after the birth[4].

Kamrin and Kamrin[5] succeeded in provoking a certain degree of organ tolerance in adult mammals by means of parabiosis, and analogous results were described by Nakic and Silobrcic[6] after skin transplantation between separated rat parabionts.

The purpose of our investigations was to determine the influence, if any, of parabiosis of long duration on the skin homografts in adult non-related mice.

Two series of experiments were performed. In the first one, white mice three months old taken from different nests were united by coelioanastomosis. After 4, 5, 6 and 7 weeks of parabiotic union, reciprocal full-thickness skin grafts between the partners were performed. The site of transplantation was the back of the animals. In the second series of experiments males of the inbred strain *C57 B1* were parabiotically united with females of the same strain. Skin was transplanted between the partners at various periods after their union. In the inbred mice of the strain *C57 B1* skin grafts between males and females always undergo destruction in the course of two weeks which probably depends on the presence of sex antigen in this strain[7]. The results obtained in the two series are summarized in the Tables 1 and 2.

During parabiosis of white non-related mice hæmolytic anæmia and the death of the anæmic partner during the first two weeks occurs in about 60 per cent of the cases[8]. Parabionts free of heavier intoxication and the usual subsequent hæmolytic anæmia can be safely kept in parabiosis for more than a year. It was found that in all pairs of white mice remaining in parabiosis for 7 weeks reciprocal 'neutralization' ensues evident in the lack of transplantation immunity and in the successful homografts. After 6 weeks of parabiotic union skin grafts only took in 50 per cent of the cases. If the parabiosis is of still shorter duration all the skin grafts undergo destruction at the same time as the grafts performed in control animals, that is, animals not parabiotically united.

In the inbred *C57 B1* mice two weeks of parabiosis was found sufficient to obtain successful skin homografts between males and females. Skin transplants on individuals of two different sexes executed just before or a few days after parabiosis are often followed by initial immunization and intensification of parabiotic intoxication. This agrees with the results obtained during parabiosis of different strains of mice[9,10].

Table 1. Fate of Skin Grafts in Parabiotic White Mice

No. of pair	Time of skin transplantation after parabiosis	Results of skin transplantation: Right parabiont	Left parabiont
7	4 weeks	−	−
33	5 „	−	−
18	6 „	−	−
24	6 „	−	+
36	6 „	+	+
37	6 „	+	−
4	7 „	+	+
15	7 „	+	+
38	7 „	+	+
40	7 „	+	+

Table 2. Fate of Skin Grafts in Parabiotic Mice *C* 57 *B*1

No. of pair	Time of skin transplantation after parabiosis	Results of skin transplantation: M	F
4	7 weeks	+	+
9	7 „	+	+
11	5 „	+	+
1	4 „	+	+
16	3 „	+	+
17	3 „	+	+
18	2 „	+	+
20	2 „	+	+
21	2 „	+	+
23	1 „	−	+

In separated parabionts both of white mice and of mice *C57 B1* none of any destructive changes in successful grafts were to be observed for 100 days since transplantation.

The results obtained in both series seem to point to the conclusion that the duration of parabiosis necessary for the induction of 'neutralization' depends on the degree of antigenic differences between the parabiotic partners. A detailed account of this work together with the results of researches now in progress relating to the possible mechanism of parabiotic 'neutralization' will be published elsewhere.

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Occurrence of *Schoenus nigricans* L. on Ombrogenous Peats

The seemingly anomalous presence of *Schoenus nigricans* on Irish blanket peats has often been reported and commented on in the literature[1,2,3].

The analysis results (Table 1) are of waters collected from a raised cupola of peat in south-west Ireland where *Schoenus* was abundant over the whole area, from the lagg region (modified by cutting) to the pools and hummocks of the apical complex. (For collection and analysis methods see Gorham[4]. Collection was made on January 15, 1959.)

The results indicate the true ombrogenity of the habitat, calcium/magnesium ratio less than unity[5], a paucity of bases and a *p*H considerably lower than that recorded for *Schoenus*-dominated blanket peats (*p*H. 5·18[5]).

The presence of this plant in such a habitat is indeed noteworthy and upholds the tentative explanation advanced by Gorham[6] that 'perhaps the mildness of the climate is the factor allowing *Schoenus* to grow in habitats less base-rich than it commonly frequents

TABLE 1

Sample area	pH	HCO^-_3	NO^-_3	Cl^-	SO^-_4	Ca	Mg	Ca/Mg	Na	K
		m. equiv./l.								
Apical complex	3·89	0	<0·002	0·762	0·140	0·055	0·174	0·316	0·539	0·017
Rand	4·02	0	<0·002	0·566	0·136	0·055	0·144	0·382	0·435	0·014
Lagg region	4·6	0·03	<0·002	0·60	0·28	0·115	0·185	0·621	0·561	0·043

elsewhere'. In Kerry, with its extremely mild climate, *Schoenus* can thrive even further from its typical ionic and *p*H optima than it can in the less-mild parts of Ireland.

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A Rapid and Simple Pathogenicity Test for Detecting Virulent Clones of *Fusarium oxysporum* f. *cubense* using Seedlings of *Musa balbisiana*

RESEARCH on *Fusarium* wilt of bananas induced by *F. oxysporum* f. *cubense* (E.F.S.) Sny. and Hans. has frequently been hindered by the lack of a rapid and simple pathogenicity test. It is well known[1,2] that parasitic isolates of *F. oxysporum* from soil and roots cannot be distinguished from saprophytes except by pathogenicity tests which are a prerequisite to placing isolates of *F. oxysporum* in their respective formæ. Recently a relatively simple field or large tank pathogenicity test was developed for *F. oxysporum* f. *cubense*[3,4]. However, this test utilized large banana plants growing in the field or in large containers and required 6–16 weeks for results. Similar tests carried out in 12-in. pots containing small banana rhizomes artificially inoculated were also satisfactory, but greenhouse shelf space limited the number of replications and the rhizomes were frequently attacked by *Pectobacterium carotovorum*[5]. In addition, above-ground symptoms usually were not evident and the plants soon became pot-bound.

In the course of studies on the pathogenicity of *F. oxysporum* f. *cubense* to seedlings of non-edible diploids of *Musa* spp., it was surprising that 3-month old seedlings of *Musa balbisiana* Colla succumbed to disease, since mature *M. balbisiana* growing wild in Honduras was known to be immune to rhizome infection even when infection was attempted artificially. Unknown to me, Umali *et al.*[6] in the Philippines had shown that the 'Pacol' clone of *M. balbisiana* succumbed to inoculations with *F. oxysporum* f. *cubense* from *M. textilis*. Other species of diploid seeded *Musa* were tested for susceptibility in Honduras. Seedlings of *M. schizocarpa* Simmonds, *M. textilis* Née, and *M. acuminata* Colla subspecies *banksii* Simmonds also succumbed to disease as a result of artificial inoculations with *F. oxysporum* f. *cubense* from 'Gros Michel' bananas. *M. balbisiana* was chosen for further study because an abundant supply of apparently homozygous seed was available from plants that escaped after introduction and were growing in the wild, isolated from other *Musa* spp. *M. textilis* was growing in stands of mixed cultivated varieties and was probably too heterozygous for consistent results. *M. textilis* and *M. balbisiana* readily hybridize. A constant and abundant seed supply of *M. schizocarpa* and *M. acuminata* subspecies *banksii* was not available.

Eight different clones of *F. oxysporum* f. *cubense* pathogenic to *M. acuminata* (AAA) 'Gros Michel' or *M. acuminata* x *M. balbaniana* (ABB) 'Chato' or 'Bluggoe'[4] and five different clones of *F. oxysporum* known to be non-pathogenic to *Musa* and unidentified as to formæ were used for inoculation. *M. balbisiana* seedlings were grown in flats of loam soil previously fumigated with methyl bromide, and were transplanted one to a 6-in. pot when about 2–3 inches tall. When approximately 5–6 inches tall, or about 3 months old from time of seeding, all plants were inoculated by placing 2 gm. of a 2-week-old 10 per cent corn-meal-in-sand culture in a trench about ½-in. deep and ½-in. from the base of the rhizome and replacing the excavated soil over the inoculum. 10 or 15 plants were used for each clone. Fungus-free corn-meal-in-sand was used on the control plants. The inoculated plants were placed in an open greenhouse at fluctuating diurnal air temperatures of 70°–98° F. and watered daily except Sundays. Above-ground symptoms consisting of leaf rolling and drooping of the older leaves followed by leaf necrosis began to appear on some of the plants 3–6 weeks after inoculation. 4–6 weeks after inoculation, all plants were dug and the rhizomes cut open. In all trials an average of 40–90 per cent of the plants inoculated with *Fusarium* clones pathogenic to 'Gros Michel' or 'Chato' bananas showed pronounced vascular discoloration of the rhizomes with or without above-ground symptoms. None of the clones non-pathogenic on 'Gros Michel' or 'Chato' induced vascular discoloration in *M. balbisiana* seedlings.

Pathogenicity studies with plants of different ages and sizes indicated that as the plants increased in size and age beyond 3 months from time of seeding, they became more resistant to rhizome infection, and above-ground symptoms seldom appeared even when the rhizome was infected. It was not determined if larger quantities of inoculum would increase the amount of disease in the larger and more mature seedlings.

All *Fusarium* clones that induced vascular discoloration in the rhizomes were readily recovered by plating the infected tissue on agar media. However, caution was indicated in placing pots containing different clones and the controls too close together on the bench as evidence of contamination from one pot to another was obtained in a few experiments.

The advantages of utilizing *Musa balbisiana* seedlings as a test for detecting virulent clones of *F. oxysporum* f. *cubense* are as follows: (1) an abundant supply of apparently homozygous seed is readily available as several pounds are obtained from one bunch of fruit; (2) the seedlings are easy to grow in small containers in both temperate and tropical climates; (3) pathogenicity testing is relatively rapid (3–6 weeks); (4) seed can be more readily shipped and

safely handled in compliance with quarantine regulations than can fleshy rhizomes or underground plant parts. This permits participation in *Fusarium* wilt research by institutions in temperate climates where an abundant supply of banana rhizomes is not available. The only disadvantage encountered thus far is that occasional lots of seed fail to germinate or have a low percentage of germination. This disadvantage will probably be overcome when more is learned about harvesting and handling the seed.

An analysis of the results obtained indicates that at least 10 plants per *Fusarium oxysporum* clone should be inoculated and that plants over 3½ to 4 months old from the time of seeding apparently develop resistance and should not be used.

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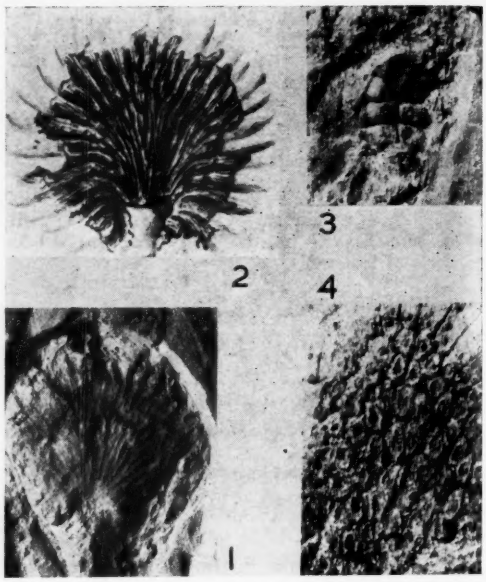

Fig. 1. *Ottokaria* cf. *bengalensis* Zeiller, from Wankie, Southern Rhodesia (× 1½). Fig. 2. *Ottokaria bengalensis* Zeiller, drawing of type specimen, from India (× 1½). Fig. 3. cf. *Scutum leslium* Plumstead, from Tangadzi River, Chiromo, Nyasaland (× 3). Fig. 4. *Scutum leslium* Plumstead (central part only) from Vereeniging, Transvaal, Union of South Africa (× 3)

PALÆOBOTANY

Occurrence of Presumed Glossopteridean Fructifications in Rhodesia and Nyasaland

The recent descriptions by Plumstead[1–3] of fructifications attached to leaves of *Glossopteris* and *Gangamopteris* found at Vereeniging in the Union of South Africa constitute an important advance in the knowledge of Lower Gondwana floras. Unfortunately, the type of preservation of the Vereeniging fossils limits the amount of information which they yield and it is therefore necessary to search elsewhere for better preserved specimens. Glossopteridean fructifications may reasonably be expected to occur in other parts of Gondwanaland. Indeed, single detached specimens, now included by Plumstead[3] in her proposed new group 'Glossopteridæ', had been described long ago from India by Zeiller[4], from Brazil by White[5], and from South Africa by Thomas[6].

Fossil plants collected by me in 1958 from various localities in the Karroo of Rhodesia and Nyasaland also include similar fructifications.

At Wankie, in Southern Rhodesia, a new quarry in the Upper Wankie (Ecca) Sandstone has yielded nine detached specimens which can be assigned with some degree of confidence to the genus *Ottokaria* Zeiller[4]. One of the better preserved specimens is illustrated in Fig. 1. It has the form of a convex round scale, about 2 cm. in diameter, surrounded by sub-acute teeth and crossed by branching striations which radiate from a presumed point of attachment near one side. This specimen shows a considerable resemblance to *Ottokaria bengalensis* Zeiller (Fig. 2), from which it differs chiefly in its rather smaller size and in the absence of a stalk, unfortunately not preserved. It should be noted that the figure of *O. bengalensis*, reproduced here for comparison with the Rhodesian specimen, is from a drawing by Seward and Sahni[7] and tends to exaggerate the clarity of the radiating striations. Zeiller's[4] original photographs look more like the Wankie specimen. Other more fragmentary specimens from Rhodesia have features recalling *O. transvaalensis* described by Plumstead[3] from Vereeniging.

In the Lower Shire River region of Nyasaland shales of Ecca age in a natural exposure on the Tangadzi River, near Chiromo, have yielded a single fragmentary specimen (Fig. 3) which probably represents part of the central portion of a fertile valve of the genus *Scutum* Plumstead[1,2]. The fragment consists of a compact group of nine swollen bodies, each 1–2 mm. in width. These bodies are arranged in the form of a shallow depression, rising from the matrix at the sides, so that no more could be exposed by development of the specimen. The central part of a specimen of *Scutum leslium* from Vereeniging is reproduced for comparison (Fig. 4) and it will be seen that the swollen bodies agree well in size, in varying shape, and especially in the possession of a small circular depression with raised central boss. The presence of *S. leslium* in Nyasaland is strongly suggested, but it must be admitted that one fragmentary specimen is scarcely sufficient to establish it beyond doubt.

The occurrence of these fructifications in the Central African Federation is a considerable extension of their range in Africa. Unfortunately, the material found so far is not better preserved than that at Vereeniging and does not throw any fresh light on the true nature of these problematical fossils. There is, however, reason to believe that further collecting in the Federation will yield more useful material, for the discovery of nine specimens at Wankie during a short period of field-work indicates that the fructifications are reasonably abundant.

A full account of these and other Karroo fossils from Rhodesia and Nyasaland will be published elsewhere.

Acknowledgement is made to the Director of the Geological Survey of India for permission to reproduce the figure of *Ottokaria bengalensis* and also to Mr. S. F. Le Roux, of Vereeniging, for providing the specimen of *Scutum leslium*.

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Note added in proof. Since the note above was submitted, an examination of leaf material from the Sebungwe River area of Southern Rhodesia has yielded well-preserved cuticles, thus confirming the belief that further field-work in the Federation will have promising results. Nov. 13.

CYTOLOGY and GENETICS

Recombinational Lethals in a Polymorphic Population

Thoday and Boam[1–3] have shown that disruptive selection can render a population of *Drosophila melanogaster* effectively polymorphic for factors affecting sternopleural chaeta number. That such selection might produce polymorphism was predicted by Mather[4]. Thoday and Boam[3] found that the polymorphism they had established depended upon a second-chromosome giving low chaeta numbers and a third-chromosome giving high chaeta numbers, each of these being balanced against chromosomes giving intermediate chaeta numbers. We have carried out further experimental analysis of second-chromosomes extracted from these polymorphic populations with remarkable results which, though there is much further work to do, seem to us worth immediate report.

The chromosomes studied were extracted from low chaeta-number flies from the experimental[1–3] population D^+. These flies were test-crossed to a 'standard' stock homozygous for the marker genes[3] *y*, *bw* and *st*. The test-cross results agreed with the previous conclusions[3] that such low chaeta-number flies were heterozygous for 'intermediate' and 'low' second-chromosomes. The extracted chromosomes were, thereafter, carried by perpetual test-cross *y*/*y bw*/*bw st*/*st* ♀ × *y* +/*bw st*/*st* ♂.

Such extracted chromosomes, by more or less usual *Drosophila* balancer techniques (using *CyL* as balancer), have been combined in various ways and resultant flies have been crossed to the *y bw st* standard to determine the viability of homozygotes, and the means and variances in the progeny of females homozygous for an extracted intermediate chromosome, or heterozygous for separately extracted intermediate chromosomes.

It has proved impossible to obtain flies homozygous for extracted low chromosomes, or flies combining any two of the separately extracted low chromosomes tested so far. The low second-chromosomes that, when combined with intermediates, determine the low chaeta numbers in the polymorphic population are therefore homozygous lethal, and the separately extracted low chromosomes are genetically similar.

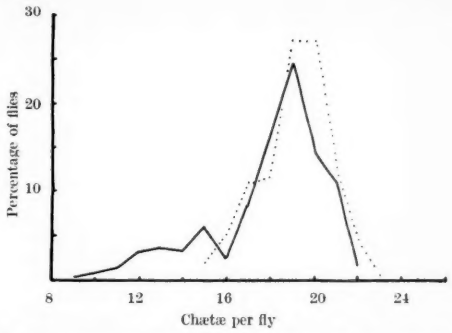

Fig. 1. Distribution curves of chaeta number for the progeny of females homozygous for an extracted intermediate second-chromosome (dotted curve) and of females heterozygous for two separately extracted intermediate second-chromosomes (solid curve). The females were crossed to a standard *y bw st* strain.

Apart from one, which we at present suspect of having carried an irrelevant lethal mutation of recent origin (it is not lethal in combination with extracted low chromosomes), the intermediate chromosomes tested have proved viable when homozygous. Progeny of females homozygous for such chromosomes crossed to the standard *y bw st* stock have given mean chaeta numbers of the order of 19 with variances about 2·0. Similar progeny of females in which two separately extracted intermediate chromosomes are combined have given means of the order of 18, and variances about 5, indicating that the differently extracted intermediate chromosomes differ at loci that readily recombine to give new chromosomes with varying effects on chaeta number. The distribution of chaeta number for the progeny of these two kinds of cross are given in Fig. 1. It seems clear that recombination gives rise to new low chromosomes. There is no evidence, either in the polymorphic population or in Fig. 1, of corresponding high chromosomes such as one would expect to be produced as reciprocal recombinants.

The low recombinant chromosomes obtained in these crosses were tested in the same manner as the low chromosomes originally extracted from the population. They too proved homozygous lethal. These new recombinant lethals were therefore combined with original lethals. These combinations were also lethal. It follows that these recombinants are genetically like those which maintain the low bristle numbers in the population.

The second-chromosome polymorphism in the population from which these chromosomes were extracted therefore involves a test-cross system of the kind +−/−+ ♀ × −−/+− where the −− chromosome has a dominant effect reducing sternopleural chaeta number and also a recessive lethal effect. In addition the population has set up a heterozygous system of intermediate chromosomes, represented by +−/−+, which not only ensures that there are intermediate second-chromosomes that can exchange from low chaeta number to high chaeta number flies (possessing high third-chromosomes), but is also so balanced that −− chromosomes are perpetually reconstituted. This, presumably, ensures effectively against the loss of such chromosomes whenever the selection imposed fails to pick them up. That a very small population could set up such a system seems a remarkable testimony to the virtuosity of the genetic system available in the wild stock from which the polymorphic population was produced, though the types of chromosome here demonstrated cannot have been present as such in the

foundation stock, for it took 13 generations of selection before the polymorphism began to develop[1,3].

The evidence put forward also seems to demonstrate that recombination was involved in the establishment of this polymorphism as has already been suggested[3] and as Mather's[5] genetic models would imply.

At the same time the population provides a nice example of a situation in which a recessive lethal can be produced by recombinational instead of mutational change.

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Cytological Identification of Proliferating Donor Cells in Chick Embryos injected with Adult Chicken Blood

The enlargement of the embryonic chick spleen which accompanies chorio-allantoic grafting of adult chicken spleen was first described by Murphy[1] in 1916. This phenomenon attracted the attention of a number of workers who believed that the splenomegaly was brought about by a proliferation of embryonic cells in response to some stimulus provided by the implanted spleen[2,3]. Simonsen[4] in 1957 showed that the splenic enlargement also occurred following the intravenous injection into the embryo of adult chicken blood or spleen cells. He showed that most embryos injected with homologous adult blood died of hæmolytic anæmia and the results of Coombs' tests strongly suggested that antibodies against the host red blood cells were present. He came to the conclusion from the results of his experiments that proliferating donor cells were responsible for the antibody production and the splenic enlargement.

It is generally agreed that the fifth largest chromosome is paired in the male domestic chicken and single in the female[5,6] and this is supported by our own observations on embryonic gonads and spleens. We have used this difference to identify male cells in the spleens of female chick embryos inoculated with cockerel blood. We have thus been able to provide a direct demonstration of the presence of cells of donor origin in the embryos.

Chick embryos of 14 days' incubation were inoculated intravenously with 0·1 ml. of citrated cockerel blood and were killed four days later. In the later experiments the colchicine-like action of demecolcine was used to increase the number of mitotic figures and to assist spreading of the chromosomes. This drug was given by injection into the allantoic cavity three hours before killing the embryos. The most satisfactory dose of demecolcine was 0·05 mgm. per embryo. The enlarged spleens from the female embryos were chopped up with scissors and the fragments incubated in half-strength Hanks' saline or 1·12 per cent trisodium citrate solution for 20 minutes at 37° C. The splenic fragments were handled as a suspension in centrifuge tubes and stained with Feulgen's reagent. The stained tissues were then examined by the squash technique[7]. No attempt was made to count the total number of chromosomes in the cells studied since identification of the sex of the cells depends upon the recognition of the paired or single

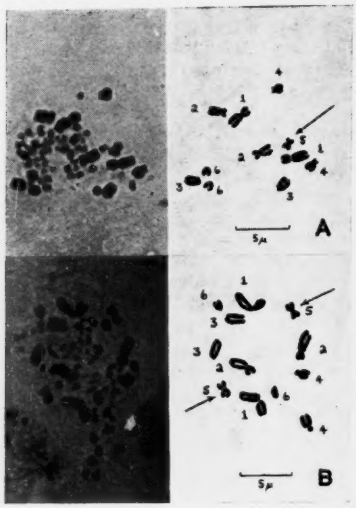

Fig. 1. Photographs with drawings of two cells in metaphase from the spleen of an 18-day-old female embryo inoculated at 14 days old with cockerel blood. The six largest pairs of chromosomes only are shown in each drawing. *A*, Female cell with single fifth chromosome; *B*, male cell with paired fifth chromosome

state of the fifth metacentric chromosome. Cells in which the 5 or 6 largest pairs of chromosomes could be identified were considered to be intact, and those with a paired fifth chromosome were identified as male cells, and those with a single fifth chromosome as females.

Male cells were found in all 15 female embryo spleens examined. A total of 170 metaphases derived from the 9 female embryos treated with demecolcine were sexed, and 75 identified as male cells and 95 as female cells. Fig. 1 shows male and female cells in metaphase. The relative proportions of metaphases of each sex varied with the embryos. No cells with paired fifth chromosomes were seen in a total of 70 metaphases from the spleens of 4 control female embryos.

These results provide a direct confirmation of the presence of cells derived from the injected blood in the embryo spleens. The relatively high proportion of female metaphases in the female spleens suggests however that an appreciable component of the splenic enlargement is provided by embryo cells.

The technique of using the sex chromosome as a cell marker has considerable potentialities in working with avian tissue, and should be of value for the cytological identification of chimerism in the domestic chicken in experiments on acquired tolerance.

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